PB82-257817

Speciation of Arsenic Compounds in Water Supplies

Texas A and M Univ., College Station

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jun 82

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-82-010	2. ORD Report	3. RECIPIENT'S ACCESSION NO. PB82 257817
4. TITLE AND SUBTITLE Speciation of Arsenic Compounds in Water Supplies		5. REPORT DATE June 1982
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Kurt J. Irgolic		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Texas A&M University Department of Chemistry College Station, Texas 77843		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. R-804774010
12. SPONSORING AGENCY NAME AND ADDRESS USEPA Toxicology & Microbiology Division Health Effects Research Laboratory Research Triangle Park, North Carolina 27711		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The objectives of this project were to develop and test analytical methods that would allow the chemical form (i.e. valence state or compound) of arsenic in drinking waters to be determined, and to use the methods to analyze samples of drinking water from sources where adverse health effects in consumers had been attributed to arsenic. Analytical techniques were developed for the determination of arsenate (differential pulse polarography), for inorganic and organic arsenic compounds (high pressure liquid chromatography with graphite furnace atomic absorption spectrometry as element-specific detector) and for the detection of arsenocholine, arsenobetaine, and iodoarsines (mass spectrometry). These techniques, inductively coupled argon plasms emission spectrometry, and hydride generation/DC-helium arc emission were used for the characterization of water samples from Utah, Alaska, Antofagasta, Taiwan and Nova Scotia. The total arsenic concentrations were in the range 18 ppb to 8 ppm with arsenic/arsenate ratios between 0.007 and 3.4. No organic arsenic compounds were detected in any of the water samples. The trace elements Al, B, Ba, Ca, Cu, Fe, Li, Mg, Mn, Na, P, S, Si and Sr were present in most of the water samples. The results show that the various physiological effects observed in populations exposed to the arsenic-containing water supplies could not be caused by arsenic compounds other than arsenite or arsenate. Other trace elements acting in concert with arsenite and/or arsenate might produce these symptoms. However, sufficient data are not yet available to evaluate this hypothesis.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 124
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

108

PB82-257817

EPA 600/1-82-010
June 1982

SPECIATION OF ARSENIC COMPOUNDS IN WATER SUPPLIES

by

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R 8047 740 10

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RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

EPA Form 2250-4 (4-80)

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SPRINGFIELD, VA 22161

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of the environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory conducts a coordinated environmental health research program in inhalation toxicology, genetic toxicology, neurotoxicology, developmental and experimental biology and clinical studies using human volunteer subjects. These studies address problems in air pollution, water pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides and other chemical pollutants. The Laboratory participates in and provides data for the development and revision of criteria documents on pollutants for which national ambient air quality and water quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U. S. inhabitants.

This report contains a description of methods that can be used to determine the specific arsenic ions and compounds that may be present in drinking waters and have heretofore been reported as total arsenic. It also contains the results of the application of these methods to samples of several drinking waters known to contain arsenic, which in earlier studies has been implicated as the cause of disease in consumers of these waters.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

The objective of this project was to determine qualitatively and quantitatively the arsenic compounds present in drinking water supplies which are known to have caused adverse health effects.

Water samples from Hinckley, Utah; Delta, Utah; the Barefoot Site, Alaska; the Mauer Site, Alaska; Antofagasta, Chile; Yenshei, Taiwan; and two sites in Nova Scotia were analyzed for total arsenic, arsenite, arsenate, arsenic compounds reducible to methylarsines and other organic arsenic compounds. Graphite furnace atomic absorption spectrometry, differential pulse polarography, hydride generation/DC-helium arc emission, high pressure liquid chromatography with a graphite furnace atomic absorption spectrometer as an element-specific detector (HPLC-GFAA) and simultaneous inductively coupled argon plasma emission spectroscopy (ICP) were employed as analytical techniques for the determination of total arsenic and arsenic compounds. ICP was also used to determine other trace elements in the water samples. The total arsenic concentrations ranged from 18 ppb to 8 ppm with arsenite/arsenate ratios between 0.007 and 3.4. No organic arsenic compounds reducible to methylarsine or dimethylarsine were detected by the hydride generation technique, which has a detection limit of 1 ppb for these compounds. The HPLC-GFAA system gave no indication of the presence of methylated or other organic compounds. The following trace elements were found in almost all water samples: Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S (probably present as sulfate), Si and Sr.

Various physiological effects (melanosis, keratosis, skin cancer, vascular ailments, pneumonia) have been attributed to arsenic. These effects cannot be caused by arsenic compounds other than arsenite and/or arsenate. The presence of arsenite and arsenate in different ratios in the water samples or one or more of the other trace elements acting in concert with arsenite and/or arsenate might produce the various symptoms. Sufficient data are not yet available to evaluate these hypotheses.

The water samples from Yenshei, Taiwan, were investigated to determine the nature of the dissolved fluorescent compounds. High pressure liquid chromatography employing UV- and fluorescence-detectors indicated that D-lysergic acid and/or ergometrine, ergotamine and perhaps calciferol might be present in these water samples. Several unknown, UV-detectable and fluorescent compounds were also found in these samples. These results call for a reevaluation of the long-held belief that arsenic alone caused the adverse health effects in the population in Taiwan exposed to these waters.

As part of this project the following techniques were developed for the

determination of arsenic compounds: differential pulse polarography for arsenate; a high pressure liquid chromatography system with a Hitachi Zeeman graphite furnace atomic absorption spectrometer as an element-specific detector for arsenate, arsenite, methylarsonic acid, dimethylarsinic acid, arsenocholine and arsenobetaine; mass spectrometry for organylarsonic acids, diorganylarsinic acids, organyldiiodoarsines, diorganyliodoarsines, arsenocholine and arsenobetaine.

This report was submitted in fulfillment of Grant No. R 8047 740 10 by Texas A&M University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period November 1, 1976 to February 28, 1981, and work was completed as of March 31, 1981.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

A -- ampere
AA -- atomic absorption
AAS -- atomic absorption spectrometry
AC -- alternating current
Ag-DDC -- silver diethyldithiocarbamate
conc. -- concentrated
DC -- direct current
DMAA -- dimethylarsinic acid
DPP -- differential pulse polarography (polarographic)
g -- gram
GFAA -- graphite furnace atomic absorption spectrometry (spectrometer, spectrometric)
HG -- hydride generation
HPLC-GFAA -- high pressure liquid chromatography-graphite furnace atomic absorption spectrometer system
ID -- inner diameter
ICP -- inductively coupled argon plasma emission spectrometry (spectrometer, spectrometric)
L -- liter
M -- molar
mA -- milliampere
MAA -- methylarsonic acid
MeOH -- methanol
mg -- milligram
min -- minute(s)
mL -- milliliter
nm -- millimeter ($1x10^{-3}$ meter)
μ -- micron ($1x10^{-6}$ meter), micro ($x10^{-6}$)
μA -- microampere
μC -- microcurie
μF -- microfarad
μg -- microgram
μL -- microliter
nm -- nanometer ($1x10^{-9}$ meter)
OD -- outer diameter
ppb -- part per billion; the concentrations of arsenic compounds are expressed in terms of arsenic, e.g., 1 ppb As(arsenate) means that the solution contains 1 ppb of arsenic in form of arsenate
pH -- negative logarithm of hydrogen ion concentration

pK_a -- negative logarithm of the acid dissociation constant
ppm -- part per million (see ppb for note)
psi -- pounds per square inch
RI -- refractive index (detector)
TBAP -- tetrabutylammonium phosphate
THAN -- tetraheptylammonium nitrate
UV -- ultraviolet
V -- volt
W -- watt

ACKNOWLEDGMENTS

The assistance of the following individuals in collecting water samples is gratefully acknowledged: Mr. E. Western, Utah State Department of Health, Division of Environmental Health (samples from Hinckley and Delta, Utah); Dr. J. M. Borgono, Servicio Nacional de Salud, Ministerio de Salud Publica, Santiago, Chile; Mrs. Alicia Araya Fuentes, Acting Director, Laboratorio Bromatologico, Antofagasta, Chile; Mrs. Stefania Razmilio Valdes, Antofagasta, Chile (samples from the Antofagasta drinking water supply); Ms. Jane L. Valentine, Associate Professor of Public Health, University of California, Los Angeles (Antofagasta water samples); Mr. Pete McGee, Alaska Department of Environmental Conservation, Fairbanks (Alaska water samples); Dr. W. Tseng, Department of Medicine, National Taiwan University, Taipei (Pu-Tai and Pei-Men samples); Dr. Fung-Jou Lu, Associate Professor of Biochemistry, College of Medicine, Taipei (Yenshei samples); and Mr. C. E. Tupper, Administrator, Environmental Health, Department of Public Health, Nova Scotia (Nova Scotia samples).

The following postdoctoral associates and graduate students participated in the analytical work: Dr. R. Bess, Mr. R. Nichols (differential pulse polarography); Dr. M. West (graphite furnace AA); Mr. D. Aylmer (adsorption of arsenic compounds by container walls); Mr. R. Stockton (HPLC-GFAA system, ICP, arsenocholine/arsenobetaine separation); Dr. P. Clark and Ms. P. Micks (hydride generation); Mr. K. Ehrhardt (mass spectrometry, arsenite/arsenate/methylarsonic acid/dimethylarsinic acid separation) and Dr. D. Chakraborti (graphite furnace AA).

Special thanks are owed to Mr. D. Heinrich, Radian Corporation, Austin, and Ms. Denise Painchaud, Applied Research Laboratories, Sunland, California for performing ICP analyses on the water samples.

Dr. I. Warner, Assistant Professor, Department of Chemistry, Texas A&M University and Mr. Dennis Shelly carried out the work on the fluorescent compounds in the Taiwan well water.

Elsevier Scientific Publishing Company, and Gordon and Breach Science Publishers granted permission to use figures from references 57 and 58 and quote passages pertinent to these figures.

SECTION 1

INTRODUCTION

Arsenic is an element which possesses a rich chemistry. Inorganic and organic arsenic compounds may contain trivalent or pentavalent arsenic. The trivalent arsenic compounds are generally more toxic than the pentavalent derivatives (1). Many inorganic and organic arsenic compounds are linked in a cycle with chemical and biologically mediated reactions changing the compounds into each other. The input of arsenic into this cycle is supplied by weathering of arsenic-containing rocks and human use and disposal of various arsenic compounds.

Since arsenic is ubiquitous, man consumes small amounts of arsenic compounds with the food he eats and the water he drinks. Life developed in the presence of arsenic. Organisms are, therefore, expected to tolerate a certain, not yet clearly defined dosage of arsenic. Certain geographically limited groups of people have taken into their systems arsenic compounds present in their drinking water supplies over extended periods of time. The most famous localities where arsenic-containing water has been consumed are certain regions in Taiwan (71-78) and the city of Antofagasta in Chile (69). Hyperpigmentation, skin cancer, vascular problems and other ailments have been attributed to the arsenic present in the drinking water. Other groups, for which the people of Fallon, Nevada, may serve as examples, have been exposed to similar arsenic levels in their drinking water without any ill effects (82). This project was undertaken in order to determine the arsenic compounds and other trace elements present in arsenic-containing water supplies and to check whether these drinking water supplies contain the same or different arsenic compounds.

SECTION 2

CONCLUSIONS

As the result of this project, together with previously developed analytical techniques, reliable methods are now available to determine arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, methylarsines, arsenocholine and arsenobetaine at the low ppb level. The high pressure liquid chromatography system employing a Hitachi Zeeman graphite furnace atomic absorption spectrometer (HPLC-GFAA) as a sensitive, element-specific detector is specially useful for the detection of yet unidentified arsenic compounds present in water samples. In order to increase the confidence in and the reliability of the analytical data, total arsenic concentrations and the concentrations of individual arsenic compounds must be determined by at least two independent methods.

The analyses of water samples from Taiwan, Antofagasta, Alaska, Utah and Nova Scotia produced total arsenic concentrations in the range 18 ppb to 8 ppm. The arsenite/arsenate ratios were in the range 0.007 to 3.4. No indications of the presence of methylated arsenic compounds, reducible to methylarsine or dimethylarsine, at levels above 1 ppb have been found. Experiments with the HPLC-GFAA system showed only the presence of arsenite and arsenate in the water samples. Most of the water samples contained Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S (probably as sulfate), Si and Sr (determined by ICP).

Various physiological effects observed in populations exposed to arsenic-containing drinking water supplies could have been caused by the presence of varying amounts of arsenite and arsenate. It is also conceivable that one or more of the trace elements present in the water supplies acted in concert with arsenic to cause the observed effects. Many more samples need to be analyzed and the results of these analyses correlated with epidemiological studies before a definite statement can be made about the interactions of trace elements with arsenite or arsenate.

The chromatographic work on the fluorescent compounds in the Taiwan well waters strongly suggests the presence of alkaloids such as D-lysergic acid, ergometrine and calciferol. These findings call for a reexamination of the presumed cause-effect relationship between arsenic and vascular diseases in Taiwan.

SECTION 3

RECOMMENDATIONS

The availability of the hydride generation technique, differential pulse polarography, high pressure liquid chromatography coupled to sensitive, element-specific detectors and colorimetric methods for the determination of total arsenic concentrations and concentrations of arsenic compounds, and the availability of simultaneous, inductively coupled argon plasma emission spectrometers for trace element determinations and of ion chromatography for anion analyses make the thorough characterization of water samples a relatively easy and not too-time-consuming task. Many more arsenic-containing water samples must be analyzed in support of or in preparation for epidemiological studies.

Arsenic has been declared a priority pollutant. Many environmental samples will have to be analyzed for arsenic compounds before final conclusions can be drawn about the health effects of arsenic compounds. Experience has shown that analysis by one method is not sufficient to produce reliable results. At least two independent methods should be used for the determination of arsenic compounds.

SECTION 4

ARSENIC COMPOUNDS IN NATURAL WATER SAMPLES

Arsenic is associated with igneous and sedimentary rocks in form of inorganic compounds. The average concentration of arsenic in these rocks varies between 0.1 ppm and several hundred ppm (1). Arsenic is found in high concentrations in sulfidic ores. Special conditions created by recent or geologically ancient volcanic or hydrothermal activities have also caused high concentrations of arsenic in localized areas. Weathering of arsenic-containing rocks produces arsenic trioxide, arsenites and arsenates which might be retained in soils or dissolved in water and transported to the ocean. In the absence of living matter water most likely will contain only arsenite and/or arsenate.

Many reactions are known which produce organic arsenic compounds from inorganic arsenic compounds (2). Such reactions generally do not occur in aqueous systems unless organisms capable of transforming arsenic compounds are present. The conversion of inorganic to methylated arsenic compounds mediated by various organisms is well established (3,4,5). Methylarsine, dimethylarsine and trimethylarsine may thus be formed from arsenite or arsenate. The formation of methylarsonic and dimethylarsinic acid was also observed (6). Microorganisms can also demethylate methylarsenic derivatives (7). These methyl-transfer reactions, the reduction of arsenate, the oxidation of arsenite, the reduction of methylarsenic acids to arsines and the oxidation of these arsines to the arsenic acids set up an arsenic cycle (Fig. 1). The arsenic cycle in nature is much more complex than the one shown in Fig. 1. Arsenic compounds are distributed among the various compartments of the environment including air, water, soil, sediments, rocks and plant and animals (7). Living matter seems to be a rich source of many organic arsenic compounds. Arsenic has been shown to be incorporated into both marine and freshwater organisms in the form of water and lipid soluble organic arsenic compounds (8). The suggestion was made (8) that arsenic is incorporated into lipids by replacing nitrogen in lecithins. A phospholipid containing 0.5 percent of arsenic (9), which might have formula 1 (R = H), was isolated from the alga *Tetraselmis chui*. Hydrolysis of the arsenic-containing lipid 1 would yield water-soluble arsenocholine 2 (R = H). Arsenobetaine 3, an oxidation product of arsenocholine 2 (R = H), was isolated from the Australian rock lobster (10). *Chaetoceros concavicornis* grown in arsenate-containing, axenic media is reported (11) to have synthesized O-phosphatidyltrimethylarsoniolactic acid 2 (R = COOH). The arsenic-containing sugar 4 was isolated from *Ecklonia radiata* (12). Most of these investigations employed marine organisms. It is very likely, that freshwater organisms are capable of synthesizing the same or similar arsenic compounds. The isolation and

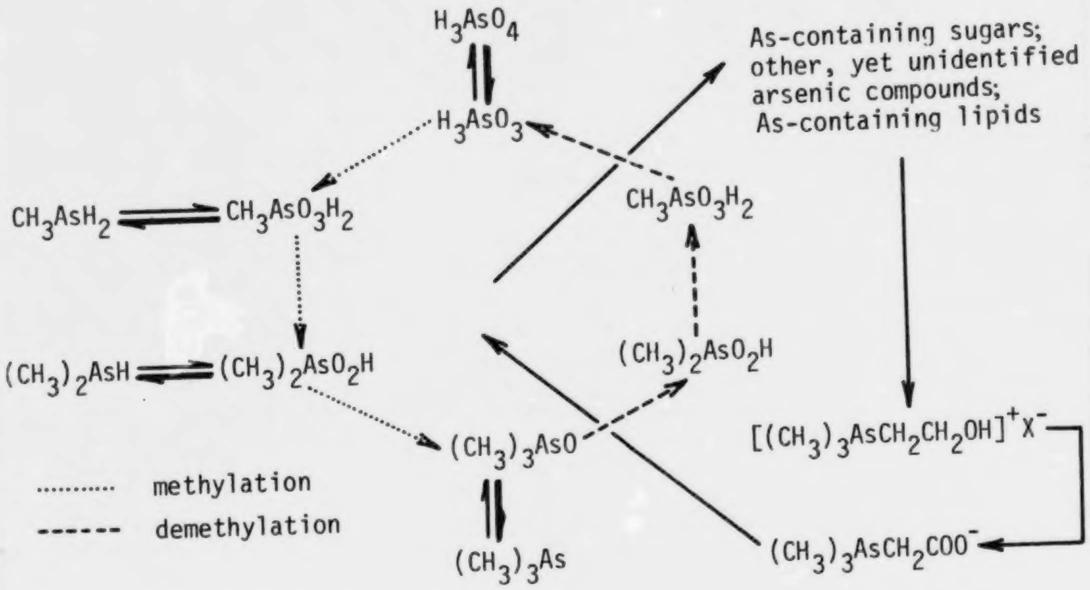
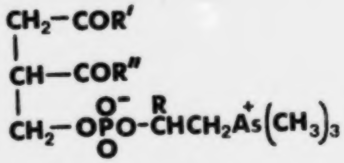
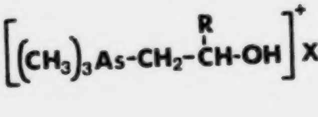

H_3AsO_4
H_3AsO_3
As-containing sugars; other, yet unidentified arsenic compounds; As-containing lipids
CH_3AsH_2
$CH_3AsO_3H_2$
$CH_3AsO_3H_2$
$(CH_3)_2AsH$
$(CH_3)_2AsO_2H$
$(CH_3)_2AsO_2H$
$(CH_3)_3AsO$
$[(CH_3)_3AsCH_2CH_2OH]^+X^-$
$(CH_3)_3As$
$(CH_3)_3AsCH_2COO^-$
methylation
demethylation

Figure 1. The transformation of arsenic compounds in the environment.



R = H, COOH

1



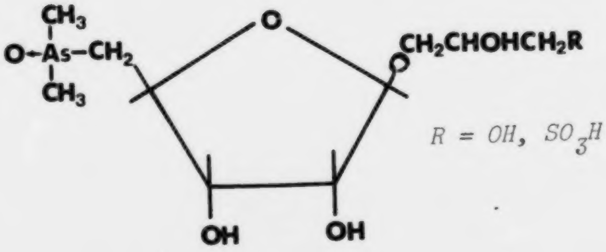
$$\left[(CH_3)_3As\text{-}CH_2\text{-}\overset{R}{\overset{|}{C}}H\text{-}OH\right]^+X^-$$

R = H, COOH

2

$(CH_3)_3\overset{+}{As}CH_2COO^-$

3



R = OH, SO_3H

4

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and identification of organic arsenic compounds has just begun. Many more, yet unknown arsenic compounds will be discovered and their properties determined as analytical techniques are improved for the speciation of such derivatives.

In natural fresh water not polluted with arsenic compounds through human activities, arsenite and/or arsenate are expected to be found. Based on the current knowledge of the arsenic cycle in nature methylarsenic compounds, arsenobetaine and arsenocholine might also be present.

Man-made arsenic compounds are used as pesticides, herbicides, desiccants, wood preservatives, feed additives and drugs (13). Most of these compounds are either inorganic or belong to the class of methylated arsenic derivatives and will be detected and quantitatively determined upon routine analysis. Pollution by the aromatic arsenic compounds employed as feed additivies and wood preservatives is unlikely unless there is a manufacturing or processing plant for such compounds near the origin of water supplies.

SECTION 5

PRESERVATION OF ARSENIC COMPOUNDS IN AQUEOUS SOLUTIONS

In contrast to most metal ions, which do not change valence easily and are not transformed into organic compounds in aqueous solution, arsenic possesses a complicated chemistry characterized by equilibria, steady state conditions or situations approaching such conditions between trivalent and pentavalent inorganic arsenic compounds and various derivatives containing arsenic-carbon bonds. A brief review of the chemistry of arsenic is available in the literature (14). The analysis for arsenic compounds in water samples, for which the term speciation shall be used, will correctly give the composition of the sample at the time of collection only if no arsenic compounds were lost, interconversions of arsenic compounds present in the samples did not occur and no new arsenic compounds were formed during the time between collection and analysis. The determination of total arsenic concentrations in samples requires only that no arsenic shall be lost and that the analytical techniques to be employed shall be applicable to all the arsenic compounds present in the sample.

Water samples can generally not be analyzed for trace elements immediately after collection. Several hours or more often several days elapse between collection and analysis. During this time the chemical nature of a particular trace element can change, trace elements can be lost by volatilization and/or can be absorbed on container walls. The adsorption of many metal ions (15, 16) and of phosphate ions (17) were studied but arsenic was rarely included.

Disagreements exist in the literature as to the extent of loss of arsenic from solutions stored in various containers. These disagreements are probably caused by differences in composition of the samples and sample containers, the species of arsenic present and the sensitivity of analytical methods.

Acidification of samples is usually considered necessary when trace elements are to be determined (15, 16). Several acids (nitric, perchloric, hydrochloric and acetic acid) were suggested as perservatives. Guimont and co-workers (18) mention the use of acetic acid as a preserving agent for arsenic solutions but give no details. Ray and Johnson (19) report losses of arsenic up to 70 percent within one week from seawater acidified with 9 mL concentrated hydrochloric acid per liter of sample. Talmi and Bostick (20) observed no losses of arsenate from distilled-water solutions containing 15 percent nitric acid or 5 percent perchloric acid upon storage for three weeks in polyethylene or soft glass containers. Whitnack and Brophy (21) reported no loss of arsenite from samples of well water kept for one week in polystyrene vials with snap caps of polyethylene. Al-Sibaai and Fogg (22) found that the total arsenic concentrations in dilute standard solutions containing only arsenite or arsenate (20 µg mL^{-1}) remained unchanged for at least 50 days when stored in borosilicate glass, soda glass or polyethylene in light or in the dark. Experiments with seawater containing 2 µg L^{-1} of arsenic gave different results (23). When such filtered seawater samples equilibrated with 5 µC of arsenic-74 were placed in high density polyethylene, soda glass or Pyrex bottles and stored in the dark over a period of five weeks, losses of arsenic occurred with all types of bottles (23). With soda glass bottles sixteen percent had been adsorbed by the 15th day when equilibrium was attained. The uptake by polyethylene and Pyrex bottles was complete within ten days and amounted in both cases to approximately six percent (23).

Storage of water samples in the frozen state has been recommended (16, 23, 24, 25). Harrison and co-workers (26) found that frozen solutions (1 ppm As) did not lose arsenic upon freeze-drying. The average retention was 96.3±1.8 percent. Natural water samples stored below -15° or under dry ice did not lose arsenite (27). Seawater samples kept in a refrigerator at 4° showed no measurable change in the arsenite/arsenate ratio within about ten days (28). For longer storage the samples had to be frozen quickly with crushed dry ice within 15 minutes. They could then be stored frozen. In samples frozen in a freezer, spurious losses of arsenite were observed (29).

Andreae (27) stated that mono-, di- and trimethylarsine were stable in aqueous solutions for a few days when stored in air-tight containers. Methylarsonic acid and dimethylarsinic acid were lost measurably after a period of approximately three days from untreated water samples. Acidification with hydrochloric acid to make the sample 0.05 $\underline{M}$ prevented these losses.

It is not possible to recommend at this time a method for the preservation of water samples applicable to all the various arsenic compounds which may occur in water. Not enough is known, for instance, about the adsorption behavior of inorganic and organic arsenic derivatives in the presence of other compounds generally found in water. Even less knowledge exists about the conditions under which arsenic compounds are converted to other species.

Arsenite and arsenate are rather easily changed into each other. Arsenite in natural water samples is slowly oxidized to arsenate. Acidification of the sample increases the oxidation rate (27). Fifty percent of the arsenite in an aqueous standard solution (20 µg mL^{-1}) was oxidized to arsenate after storage in a glass container for 33 days (22). Arsines were oxidized by traces of air (27). An arsenic cycle operates in nature (7) connecting inorganic with organic arsenic compounds. The chemical nature of many of the organic arsenic compounds participating in this cycle are still unknown.

EXPERIMENTAL

Conditions which will prevent adsorption are likely to favor transformation of arsenic compounds and vice versa. A universal preservation agent which would prevent adsorption and transformation is not known and will probably not be found. It will be necessary to develop preservation techniques for each sample dependent upon the type of arsenic compounds present. Investigations were carried out to find conditions under which arsenic compounds would not be adsorbed on container walls and arsenite would not be converted to arsenate.

Most of the water samples, which are the subject of these speciation studies, contained arsenic at concentrations of approximately 1 ppm. To obtain data on the amount of arsenic lost to container walls, the adsorption of arsenate, arsenite and dimethylarsinic acid from aqueous 1 ppm As solutions on polyethylene and Pyrex was studied.

New polyethylene Cubitainers (soft polyethylene manufactured by Kimberly) and Pyrex glass containers were filled with 7.5 $\underline{M}$ nitric acid and kept for 24 hours. They were then emptied and rinsed thoroughly with deionized water. The arsenite and arsenate solutions were prepared using either distilled, deionized water of pH 6.5 or tap water of pH 8 with a sodium concentration of 211 ppm. Neither water contained arsenic.

Weighed quantities (required for the preparation of stock solutions) of $Na_2HAsO_4 \cdot 7H_2O$, $NaAsO_2$ or As_2O_5 were irradiated with neutrons at the Texas A&M University Nuclear Science Center to produce ^{76}As, a gamma-emitter with a half life of 24.6 hours. For the experiments with Pyrex flasks the stock solution was prepared by dissolving irradiated As_2O_5 in distilled water. The stock solutions for the polyethylene experiments were made from $Na_2HAsO_4 \cdot 7H_2O$ or $NaAsO_2$. Working solutions of 1 ppm arsenic (As) were prepared by appropriate dilution.

Acid-cleaned 25 mL volumetric flasks made from Pyrex were filled to the mark with the 1 ppm As solution (prepared from As_2O_5). One flask each was emptied after one, two, four, five, six and ten days had elapsed. The flasks were rinsed, then filled to the mark with distilled water and placed into the gamma-ray counter well equipped with a Li-drifted germanium detector. The entire gamma spectrum was recorded. The areas under the arsenic peaks were computed and corrected for the decay of the ^{76}As-isotope. A calibration curve was obtained by counting 25 mL-volumetric flasks containing 0.5, 1.0, 1.5 or 2.0 mL of the 1 ppm As solution in 25 mL.

For the adsorption studies in polyethylene bottles, 1 mL of a solution containing 100 ppm As of either arsenate or arsenite was pipetted into 100 mL of distilled water or tap water stored in the plastic bottles. These bottles were treated and counted as described above for the Pyrex studies.

Samples of 1 ppm As solutions containing dimethylarsinic acid in polyethylene bottles were prepared similarly. The arsenic concentrations in solution as a function of time were determined by flameless atomic absorption spectrometry after a ten-fold dilution of an appropriate aliquot.

For the polarographic determination of arsenite in solution, 45 mL were withdrawn from the plastic bottles and transferred into the polarography cell. After addition of 5 mL of conc. HCl nitrogen was bubbled through the solution for five minutes before performing the analysis. To determine arsenate, 30 mL of the solution, 10 mL conc. $HClO_4$ and 3 g pyrogallol were placed into a 50 mL volumetric flask. After filling to the mark with distilled water, the cell was charged with this solution. Calibration curves were obtained by standard addition.

The following systems were investigated:

Arsenate in distilled water - Pyrex
in distilled water - Polyethylene
in tap water - Polyethylene

Arsenite in distilled water - Polyethylene
in tap water - Polyethylene

Dimethylarsinic Acid in distilled water - Polyethylene
in tap water - Polyethylene

RESULTS

When 1 ppm As (arsenate) solutions were kept in 25 mL acid-cleaned Pyrex volumetric flasks activity corresponding to 0.22 µg of arsenic was found on the walls after one day. The amount of arsenic adsorbed did not change during the ten-day experiment. The samples could not be counted after more than ten days had elapsed because of decay of the ^{76}As-isotope.

In all the experiments in polyethylene bottles no adsorption of arsenate, arsenite or dimethylarsinic acid was detected. The results for dimethylarsinic acid are tentative because the results of atomic absorption spectrometry are accurate only within ±5 percent (30). ^{76}As-Dimethylarsinic acid was not available for these experiments. After two days all the arsenite in the $NaAsO_2$ solutions had been converted to arsenate (checked by polarography). These studies indicate that polyethylene bottles are the containers of choice to store aqueous solutions of arsenite and arsenate.

Among the chemical changes to which arsenic compounds may be subjected in aqueous solutions, the oxidation of arsenite to arsenate has been reported repeatedly in the literature (7, 22, 27, 31). Preliminary analyses of water samples to be speciated for arsenic compounds indicated the presence of arsenite and arsenate. To keep these two ions from converting into each other a search for an appropriate preservation technique was made.

The most convenient and experimentally tested procedure for the preservation of arsenite was reported by Feldman (31). Ascorbic acid at a concentration of 1 mg mL^{-1} prevented the oxidation of arsenite to arsenate in aqueous standard solutions at room temperature. Arsenate was not reduced to arsenite under these conditions (31). However, special care must be taken, not to expose arsenite solutions preserved by ascorbic acid to elevated temperatures. Ascorbic acid has been used to reduce arsenate to arsenite in boiling seawater (23). Whether ascorbic acid scavenges oxygen from the solution, combines with arsenite producing a species less reactive toward oxidation than arsenite or acts both ways in preventing oxidation of arsenite, is not known. Natural water samples contain many other metal ions. Comprehensive experiments to elucidate the influence of such metal ions on the ability of ascorbic acid to preserve arsenite have not yet been carried out. Metal ions do catalyze the oxidation of ascorbic acid by oxygen (32).

The water samples which were analyzed as part of this project contained arsenite and arsenate (see Section 7). Additional water samples were analyzed during the past six months. On the basis of the experience gained with these samples the following procedure is recommended for the analysis of arsenite and arsenate in water samples:

- Collect three samples for each water supply to be analyzed in one-quart polyethylene Cubitainers.
- Preserve the first sample by addition of 0.1 percent by weight of ascorbic acid.
- Preserve the second sample by addition of 7 mL ultrapure, concentrated nitric acid to one quart of sample.
- Leave the third sample unpreserved.
- Fill all the containers to the brim to avoid air spaces.
- Analyze the samples as soon as possible after collection.
- The samples should be kept at or below room temperature during shipment and storage.

If the concentrations of arsenate and arsenite in the three samples determined by at least two independent methods agree, arsenite and arsenate were not chemically changed during the period between collection and analysis. Among the fifteen different water samples analyzed by this procedure thus far, the arsenite and arsenate values obtained from a set of preserved and unpreserved samples did not agree in only one case.

Since methylated arsenic compounds were not present in any of these samples, no statement can be made about the effect of this procedure on organic arsenic derivatives.

SECTION 6

DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF ARSENIC COMPOUNDS

Several methods are available to determine total arsenic concentrations: colorimetry (silver diethyldithiocarbamate - AsH_3 complex; arsenomolybdic acid); neutron activation analysis employing the gamma-active isotope ^{76}As; polarography of arsenite; coulometry; X-ray fluorescence spectroscopy; atomic emission spectrometry; plasma emission spectrometry; and gas chromatography of volatile arsenic compounds. Recent reviews of these techniques are available (33, 34). The detection limits may be as low as picograms of arsenic per milliliter.

Whereas many methods exist for the determination of total arsenic, the choice of techniques for the determination of individual arsenic compounds is limited. The following methods were available for the determination of arsenic compounds when this project was initiated. The arsenic compounds, determined by these methods, are listed as part of the heading. It is likely that other arsenic compounds not yet investigated may also be determinable by these methods.

Silver diethyldithiocarbamate (Ag-DDC) / colorimetry for inorganic arsenic and methylarsonic acid: Arsine generated by reduction of arsenite and/or arsenate and methylarsine obtained by reduction of methylarsonic acid, reacts with Ag-DDC in pyridine to produce colored complexes. Zinc/hydrochloric acid serves as the reducing agent. The absorbances of the complexes are measured at 540 and 440 nm. Detection limits as low as 20 ppb are achieved (35).

Ammonium molybdate for arsenite and arsenate: Arsenate (but not arsenite) reacts with ammonium molybdate to form blue arsenomolydate, the absorbance of which is measured at 865 nm. Arsenite can be oxidized to arsenate for the determination of total inorganic arsenic. Reduction of arsenate to arsenite with sodium disulfite and sodium thiosulfate in aqueous sulfuric acid allows the determination of phosphate (36).

Polarography for arsenite: Arsenite can be determined polarographically in acidic aqueous media by single-sweep polarography (21) (sulfuric acid, detection limit 5 ppb) or by differential pulse polarography (37) ($1\ \underline{M}$ hydrochloric acid, detection limit 0.3 ppb). Arsenate is polarographically inactive under these conditions. Dimethylarsinic acid, methylarsonic acid (38), and other aliphatic (39) and aromatic (40) arsinic and arsonic acids are reducible on the dropping mercury electrode. Whether inorganic arsenic compounds and organylarsenic acids can be determined simultaneously by polarography must yet be decided by experiment.

Hydride generation for arsenite, arsenate; methyl-, ethyl-, propyl, butyl-, phenylarsonic acid; dimethylarsinic acid; trimethylarsine oxide: Arsenite, arsenate, organylarsonic acids, diorganylarsinic acids and trimethylarsine oxide are reduced in aqueous medium by sodium borohydride to arsines (27, 31, 41, 42, 43). Speciation of these compounds is accomplished by their pH selective reduction to arsines, transferring the arsines into an organic solvent (43), or adsorbing the arsines on Chromosorb 101 (43), selective volatilization of arsines from a cold trap (27, 41, 42, 43) or gas chromatographic separation of the arsines (27, 43) and detection of the separated arsines by helium-DC discharge spectrometry (31, 41, 42), argon microwave emission spectrometry (43), atomic absorption spectrometry using a hydrogen/air flame (27, 44), electron capture or flame ionization detectors (27). Detection limits in the part per trillion range have been reported (27). Great care must be exercised when arsenic compounds are reduced with sodium borohydride. Some arsenate may be reduced to arsine under conditions under which only arsenite should react, and cleavage of methyl groups from methylarsenic compounds has been observed during their reaction with sodium borohydride (31). Improvements concerning the reduction conditions (31) and modifications of the analytical system (31, 45) made the hydride generation technique a more reliable method for the determination of arsenic compounds.

The disadvantages of the hydride generation method include the requirement that the arsenic compounds must be reducible to arsines, and the necessity that the arsines possess sufficient volatility to allow their transfer to the detectors. Many arsenic compounds exist which do not fulfill these requirements.

Derivatization/gas chromatography for methylarsonic acid and dimethylarsinic acid: Methods which digest samples to convert arsenic compounds to inorganic derivatives (46) and subsequently transform these into organic derivatives (20, 47) suitable for gas chromatography cannot be employed for speciations. All information about the chemical nature of the original compounds is lost during digestion. Volatile arsenic compounds, such as methylarsines, can be determined without prior chemical modification by gas chromatography (27, 48).

Chemical modification has been successfully employed to convert methylarsonic and dimethylarsinic acid to volatile derivatives. Methylarsonic acid combines with ethylene glycol (49), dimethylarsinic acid reacts with hydriodic acid (50), and both acids interact with allylthiourea (51) to form arsenic derivatives which can be determined by chromatography. The reactions of arsenic compounds with trimethylsilyl chloride produce volatile arsenic compounds. Unfortunately, the method is non-selective. Several arsenic compounds gave the same product (52).

Gas chromatography/atomic absorption spectrometry for arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide and methylarsines: Arsenic compounds, which possess adequate volatility or can be converted to volatile compounds, can be separated by gas chromatography and quantitated employing graphite furnace (53) or flame (27) atomic absorption spectrometers as element-specific detectors. This method has not been used extensively.

Gas chromatography/mass spectrometry for methylarsines and ethyl, propyl- and butylarsine: After separation of volatile arsenic compounds by gas chromatography a high-resolution mass spectrometer may serve as an element-specific and compound-specific detector which can also provide the elemental compositions for the compounds (43).

All the methods available for the speciation of arsenic compounds at the time this project was initiated had severe limitations. The methods were applicable only to the determination of a few arsenic compounds or required their conversion to volatile derivatives. Practically, the quantitative determination of arsenic compounds was thus limited to arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, ethyl-, propyl- and butylarsonic acid, and the arsines obtainable from these compounds. Non-volatile arsenic compounds and arsenic compounds not reducible to volatile arsines could not be determined with the existing methods. Therefore, an analytical system with an element-specific detector had to be developed capable of separating volatile and non-volatile arsenic compounds from complex matrices.

The development efforts produced a high pressure liquid chromatography - Hitachi Zeeman graphite furnace atomic absorption system, a differential pulse polarographic method for the determination of arsenite and arsenate, and the elucidation of the mass spectral behavior of organylarsenic acids, organyliodoarsines, arsenocholine and arsenobetaine.

HITACHI ZEEMAN GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETER AS AN ELEMENT-SPECIFIC DETECTOR FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY

Liquid chromatography and specially high pressure liquid chromatography (HPLC) with the great resolving power of its micro-particulate column materials are potentially the best techniques for the separation and simultaneous determination of arsenate, arsenite and organic arsenic compounds. A water sample may contain many substances in addition to arsenic compounds, which can be separated by HPLC techniques. The common detectors (refractive index, UV-visible spectrometer) will not respond specifically to arsenic compounds. The identification of arsenic-containing fractions is, therefore, difficult if not impossible unless element-specific detectors with high sensitivity are available.

Flame atomic absorption spectrometers have been interfaced with chromatographic columns for the element-specific detection of several metal ions (54). The detection limits were relatively poor primarily because of peak spreading in the chromatographic column. Cantillo and Segar described a system in which the effluent flow is stopped at fixed time intervals for introduction of a sample into the graphite furnace (55). This system gave better sensitivity but the total analysis time was very long. A graphite furnace atomic absorption spectrometer (GFAA) combines the advantage of element-specificity with high sensitivity for many elements. Two automated systems combining HPLC with GFAA as an element-specific detector were simultaneously and independently developed. The first system uses a Perkin-Elmer GFAA with a specially adapted autosampler (56). The second system, developed for this project, employs a Hitachi-Zeeman GFAA with a sample valve, an injector and associated electronics to control the analysis sequence (57).

Description of the HPLC-Hitachi-Zeeman GFAA System

In order to determine arsenic quantitatively in the HPLC column effluents an automated HPLC-GFAA interface had to be developed capable of controlling the following functions sequentially and reliably.

- Lowering the injection tube into the cool graphite cup.
- Turning the sample valve into the inject position.
- Actuating the pump to inject nickel solution into the graphite cup (if desired) and controlling time of its operation to deliver a predetermined volume of this solution.
- Turning on the Start/Stop switch to initiate the AA analysis sequence.
- Raising the injection tube out of the graphite cup and the furnace.
- Returning the sample valve to the load position in preparation for the next injection.
- Allowing the GFAA to perform the analysis and the graphite furnace to cool sufficiently to permit injection of the next sample without splattering.
- Actuating the Start/Stop switch to terminate the graphite furnace cooling cycle.
- Returning to the first function to repeat the sequence of events.

Although there are several possibilities to accomplish the required sequencing and timing of events, the design chosen divides the interface into the sampling sub-system, the injection sub-system and the control and timing circuit. A block diagram for the HPLC-GFAA system is given in Fig. 2 and schematics for the interface are presented in Fig. 3. The specifications and sources for the electronic components are summarized in Table 1.

The Sampling Sub-System: The sampling sub-system consists of an Altex, eight port, Teflon Slider Injection Valve (Altex catalog #201-14) with two attached pneumatic actuators and connecting tubings as shown in Fig. 4. One of the eight ports (Fig. 4) is closed in one of the two possible slider positions. To this port the nitrogen line is connected. When the nitrogen port is in the open position it will connect to the "bottom sample loop port" (BSLP). The BSLP is connected by a 0.6 mm ID Teflon tubing to the port directly above it (top sample loop port, TSLP). The length of the Teflon tubing is adjusted to deliver the volume (40 µL in the case of the HPLC-GFAA system

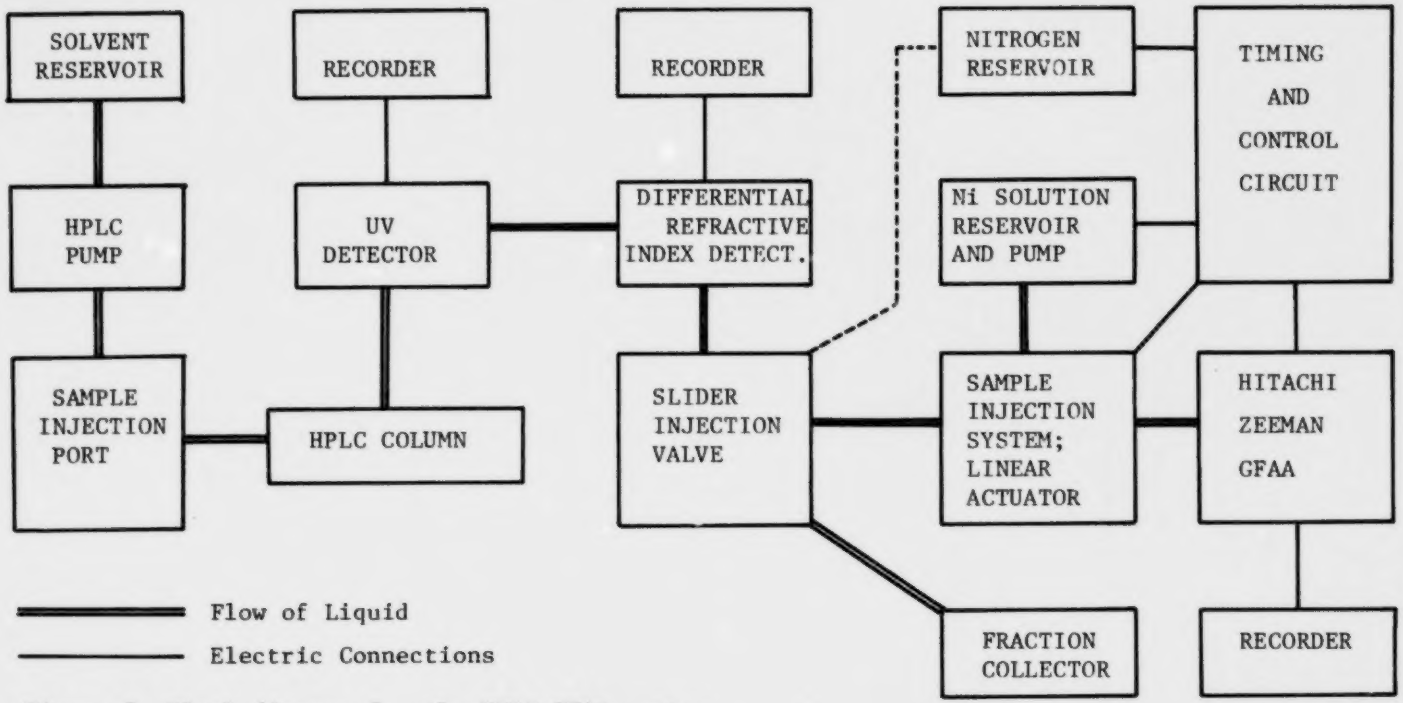
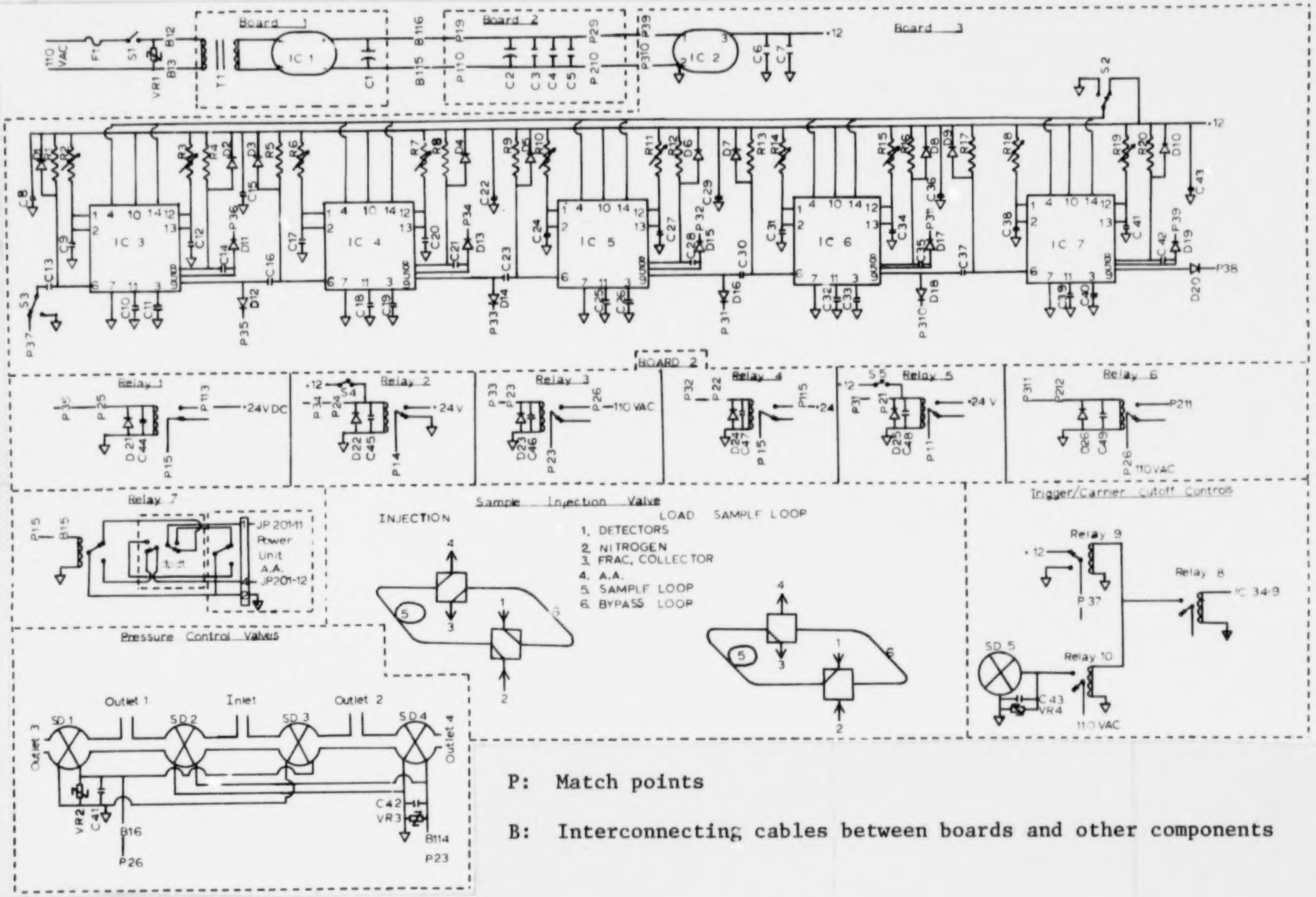

SOLVENT RESERVOIR
HPLC PUMP
SAMPLE INJECTION PORT
RECORDER
UV DETECTOR
HPLC COLUMN
RECORDER
DIFFERENTIAL REFRACTIVE INDEX DETECT.
SLIDER INJECTION VALVE
NITROGEN RESERVOIR
Ni SOLUTION RESERVOIR AND PUMP
SAMPLE INJECTION SYSTEM; LINEAR ACTUATOR
FRACTION COLLECTOR
TIMING AND CONTROL CIRCUIT
HITACHI ZEEMAN GFAA
RECORDER
Flow of Liquid
Electric Connections

Figure 2. Block diagram for the HPLC-GFAA system.

(From reference 57; redrawn with permission of Gordon and Breach Science Publishers)


Board 1
Board 2
Board 3
110 VAC
F1
S1
VR1
B12
B13
T1
IC 1
C1
B116
B115
P19
P110
C2
C3
C4
C5
P29
P210
P39
P310
IC 2
C6
C7
+12
S2
IC 3
IC 4
IC 5
IC 6
IC 7
S3
P37
P38
BOARD 2
Relay 1
Relay 2
Relay 3
Relay 4
Relay 5
Relay 6
Relay 7
Relay 8
Relay 9
Relay 10
+24VDC
+24 V
110 VAC
JP 201-11
Power
Unit
A.A.
JP201-12
Sample Injection Valve
INJECTION
LOAD SAMPLE LOOP
1. DETECTORS
2. NITROGEN
3. FRAC. COLLECTOR
4. A.A.
5. SAMPLE LOOP
6. BYPASS LOOP
Trigger/Carrier Cutoff Controls
IC 34-9
SD 5
C43
VR4
Pressure Control Valves
Outlet 1
Inlet
Outlet 2
Outlet 3
Outlet 4
SD 1
SD 2
SD 3
SD 4
VR2
C41
B16
P26
C42
VR3
B114
P23

P: Match points

B: Interconnecting cables between boards and other components

Figure 3. Schematics for the HPLC-GFAA interface.

TABLE 1. SPECIFICATIONS AND SOURCES FOR ELECTRONIC COMPONENTS FOR THE HPLC-GFAA INTERFACE

COMPONENTS DESIGNATION*	SPECIFICATIONS AND SOURCES
C 1,2	100 μF, 50 V electrolytic capacitors
C 3,7,9,22,36	0.1 μF, 500 V ceramic disc capacitors
C 4,5,15,29,43-49	0.01 μF, 1000 V ceramic disc capacitors
C 6	10 μF, 25 V tantalum electrolytic capacitors
C 9,10,11,18,19,25,26 32,33,39,40	0.01 μF, 50 V ceramic disc capacitors
C 12,17,20,24,27,31,34, 38,41	100 μF, 50 V electrolytic capacitors
C 13,14,16,21,23,28,30, 35,37,42	0.001 μF, 50 V ceramic disc capacitors
D 1-26	600 V PRV, 1.0 A silicon diodes, Sylvania model ECG116
F 1	1.5 A quick blow fuse
IC 1	30 A, 70 PRV epoxy bridge rectificer, type VK 148 distributed by Allied Electronics
IC 2	+12 V voltage regulator, type LM 340-12
IC 3-7	LM 556 CN dual monostable multivibrators (timers)
R 1,4,5,8,9,12,13,16, 17,20	10 kΩ, 1/4 W resistors
R 2	5 MΩ, 1/4 W variable resistors
R 3,6,7,10,11,14,15 18,19	2 MΩ, 1/4 W variable resistors
Relay 1-6,9,10	12 V-DC, 60 mA SPDT relays; Calectro part #974
Relay 7	SPDT mercury-wetted reed relay, series W133MPC, Allied Electronics
Relay 8	5 V-DC, 2.3 mA SPDT relay type R10S-E1-Y1J, Allied Electronics
S 1,4,5	Single pole, single throw switch
S 2,3	Single pole, single throw switch
SD 1-4	110 V-AC, 9W solenoid valves, NC model #61P18C3, Valcor Engineering Copr., Kenilworth, N.J.
SD 5	110 V-AC, 7 W three-way solenoid valve model #V55KJ8NGV, Peter Paul Electronics, New Britain, Conn.
T 1	35 V, 1.5 A Centertap transformer
VR 1-4	150 V RMS metal oxide varistors, model #750, Workman Electronics, Sarasota, Florida
dpdt	Double pole double throw switch

*Component designation as used in Figure 3.

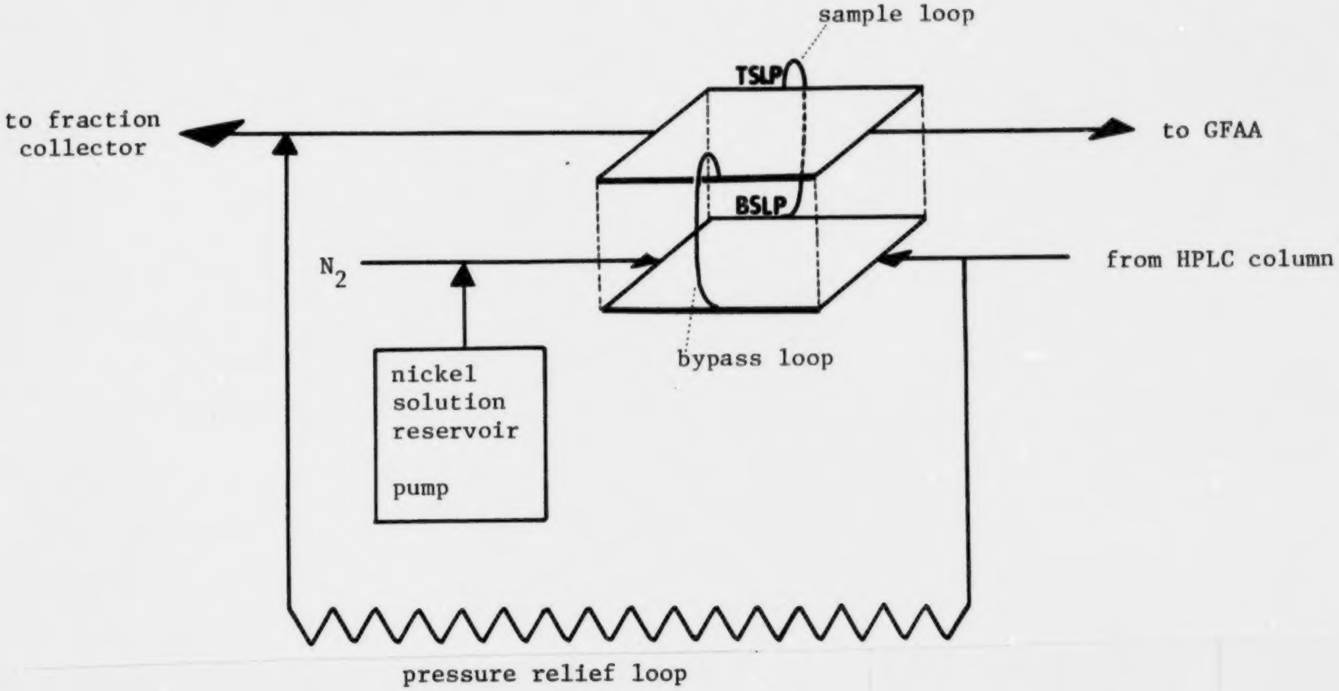

sample loop
TSLP
to fraction collector
to GFAA
BSLP
N_2
from HPLC column
bypass loop
nickel solution reservoir pump
pressure relief loop

Figure 4. The sampling sub-system of the HPLC-GFAA interface.

described here) required by the graphite furnace spectrometer. When the nitrogen port is open, the TSLP is connected to the port leading to the injection device. The bypass loop uses the two ports opposite the BSLP and the TSLP. The volume of the bypass loop should be made as small as conveniently possible. To the port opposite the nitrogen port the tubing coming from the HPLC column is connected. The port above the nitrogen port and opposite the ports leading to the injection device is the outlet port for the HPLC eluent going to a fraction collector or to waste. Several feet of a 0.3 mm ID Teflon tubing (pressure relief loop, Fig. 4) connect the tubing coming from the column with the line leading to the injector. This restrictor tubing is required for relief of any pressure built up during the time the valve switches between load and injection postions. Pressure build-up may damage the windows in the refractive index detector.

During the load cycle the nitrogen port, the bypass loop and the injection port are closed. The effluent passes from the inlet line through the sample loop to the fraction collector. The valve is switched from loading to injection by two pneumatic actuators, which are controlled by the pressure control system shown in Fig. 3. Nitrogen at 60 psi is introduced via the inlet to selenoids SD 2 and SD 3, which determine whether nitrogen is routed to outlet 1 or outlet 2. Outlets 1 and 2 are connected to the actuators on the slider injection valve. Outlets 3 and 4 are exhaust ports controlled by selenoids SD 1 and SD 4, respectively. Selenoids SD 1 and SD 3 operate simultaneously as do selenoids SD 2 and SD 4 releasing pressure from one actuator and applying it to the other thereby changing the slider injection valve from the loading to the inject position. During the injection cycle the column effluent travels from the inlet line through the bypass loop to the fraction collector. The sample contained in the sample loop is pushed out from the loop into the injection line by nitrogen pressure. The nitrogen at approximately 5 psi comes from a nitrogen tank. To deliver nickel solution to the graphite cup a peristaltic pump with a capacity of 200 µL per minute is activated for the required amount of time to push the appropriate volume of nickel solution (10 µL in the case of the HPLC-GFAA system described here) into the nitrogen line just before the nitrogen port. Nitrogen pressure then transports the nickel solution through the sample loop and the injection line into the graphite cup. The entire cycle begins again after the slider injection valve is switched back to the load position.

The Injection Sub-System: The injection system consists of an American Design Components 2-½ inch stroke 24 V-DC linear actuator, a filter capacitor (0.2 µF, 200 V), injection tubing and mounting brackets (Fig. 5). The positioning bracket is slotted for exact positioning of the injection probe on the mounting bracket. The delivery tube is a section of 1/16 inch OD 316 Stainless Steel tubing inside a section of 1/8 inch OD 316 Stainless Steel tubing (guide tube). The back support plate has a slot into which the injection bar extends holding it straight and providing stops at the top and the bottom for the actuator stroke. The mounting bracket has a viewing port through which the injector can be exactly positioned. The injection sub-system must be very carefully positioned and adjusted. The samples must be deposited into the center of the cup without splattering and without touching the sides of the graphite cup. The delivery tube must

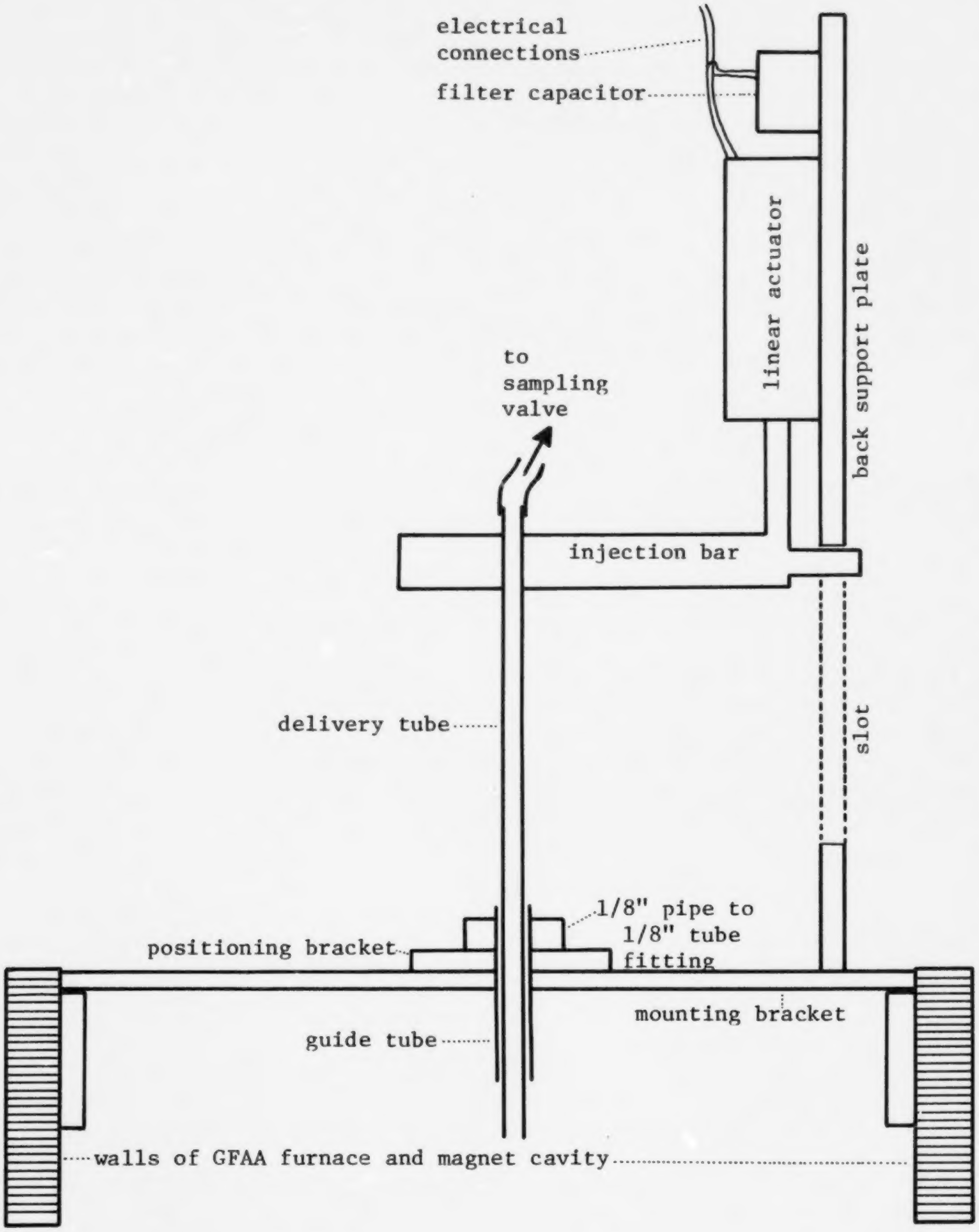

electrical connections
filter capacitor
linear actuator
back support plate
to sampling valve
injection bar
delivery tube
slot
1/8" pipe to 1/8" tube fitting
positioning bracket
mounting bracket
guide tube
walls of GFAA furnace and magnet cavity

Figure 5. The injection sub-system of the HPLC-GFAA interface.

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TOP OF IMAGE AREA
CHAPTER HEAD BEGIN SECTIONS HERE
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TYPING GUIDE SHEET

just touch the deposited sample but not the sides of the cup.

The Timing and Control Circuits: The timing and control circuits are divided into three printed circuit boards (Fig. 3). Board 1, the power supply board, consists of the transformer T 1, which converts 110 V-AC to 17.5 V-AC, and the rectifier IC 1, which changes 17.5 V-AC to 24.7 V pulsating DC. Ripple is removed by capacitor C 1.

Board 2 contains additional filtering and despiking capactitors C 2-5 and relays 1-6. The despiking capacitors remove line transients, which might damage the voltage regulator IC 2 on board 3. The SPDT relays 1-6 control the GFAA, the injector and the actuators on the slider injection valve.

Relay 1 is closed by a signal from IC 3B set at 1 second. Closing of relay 1 actuates relay 7 which is connected in parallel with the start/stop switch on the AA and thus terminates the cooling cycle, the length of which is controlled by IC 3A.

Relay 2 activated by timer IC 4A switches on the motor of the linear actuator lowering the delivery tube into the graphite cup. IC 4A is set to allow the injection bar to reach the bottom stop in the back support plate.

Relay 3 controlled by timer IC 4B activates solenoids SD 1 and SD 3 which cause the slider injection valve to change from the load to the inject position. At the same time the nickel pump is activated and kept on by IC 4B long enough for 10 μL nickel solution to flow into the injection system.

Relay 4 controlled by timer IC 5B switches on the motor of the linear actuator to raise the delivery tube out of the graphite cup. IC 5B is set to allow the injection bar to just reach the top stop in the back support plate.

Relay 6 controlled by timer IC 6A set at one second activates solenoids SD 2 and SD 4 which return the slider injection valve to the load position. The silicon diodes D 21 - D 26 and the capacitors C 41 - C 49 minimize line transients propagating into the timing circuit.

Board 3 contains the timing circuit for the HPLC-GFAA interface consisting of five cascaded LM 556 CN dual, monostable multivibrators (Fig. 3, Table 1) and one 12 V-DC LM 340T 12 voltage regulator, IC 2. IC 2 converts the 24.7 V-DC coming from Board 1 to a regulated 12 V-DC providing power for the timing circuit and the relays 1-6. Each one of the dual multivibrator packages (IC iA and IC iB; i=1-7) controls two events. The timing in each multivibrator is controlled by a capacitor and a variable resistor. This arrangement makes it possible to time each multivibrator independently of the other nine multivibrators. The first multivibrator IC 3A (Fig. 3) is triggered by relay 9, which is activated through relay 8 by the timer (IC 34-9) in the GFAA determining the length of the atomization cycle (Trigger/Carrier Cutoff Controls, Fig. 3). IC 3A remains on until the graphite cup has cooled. The output pin of each multivibrator (with the expection of the last multivibrator IC 7B) is connected to the input pin

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of the following multivibrator through a 0.001 µF capacitor. When a multivibrator has timed out, the output pin returns to ground triggering the next multivibrator. This arrangement assures that the events occur in the intended sequence, because a timer cannot start until the preceding one has timed out. The pin configurations and other pertinent specifications of the multivibrators are described in the manfacturer's literature (59).

The timers IC 6B, 7A and 7B are not utilized presently. They provide, however, the capability of dual injections of sample into the graphite cup to increase the sample size to be analyzed. The second injection will come after the drying cycle for the first injection has been completed.

The diodes D 1 - D 10 sharpen the triggering pulses to the respective timers IC 3 - IC 7. The bypass capacitors C 10,11,18,19,25,26,32,33,39 and 40 reduce the susceptibility of the timers IC 3 - 7 to noise. The despiking capacitors C 8,15,22,29,36,43 remove line transients.

The switch S 2 interrupts the timing sequence and resets all timers. The switch S 3 allows the manual starting of the timing sequence.

The Trigger/Carrier Cutoff Controls of the interface are mounted inside of the power supply of the GFAA. Relay 8 is connected to IC 34-9 in the GFAA power unit. When IC 34-9 initiated the atomization cycle relay 8 is activated, which in turn activates relays 9 and 10. Relay 9 triggers timer IC 3A. Relay 10 turns on solenoid SD 5 which terminates the carrier gas flow through the graphite furnace during the atomization cycle. Capacitor C 43 and varistor VR 4 control voltage transients.

Relay 7 is mounted outside the GFAA power unit together with the switch dpdt (double pole-double throw). This switch isolates the GFAA from the interface allowing normal operation of the spectrometer.

The HPLC-GFAA system has functioned almost flawlessly during the past three years. The major problems encountered initially were caused by line spikes propagated through the building wires to the electronics of the interface, where they triggered relays and disturbed the proper sequence of events. The capacitors installed as shown in the schematics (Fig. 3) eliminated this interference.

The time interval between two analyses can be made as short as 30 seconds. In most cases analyses carried out in one minute intervals produce satisfactory chromatograms. The firing of the graphite furnace every minute caused the permanent magnets of the GFAA unit to overheat. Therefore, a refrigerated glycol/water mixture was circulated through the magnet's cooling system replacing tap water used under normal operating conditions. Because the glycol/water mixture was pumped at much lower pressure than the tap water, the water pressure switch in the GFAA had to be deactivated. The instrument is still protected by an overheat shut down switch. The time between analyses can be further shortened by using cooled gases to bring the graphite cup to room temperature after completion of the atomization cycle and by shortening the drying cycle.

The Hitachi-Zeeman GFAA Model 170-70 has a detection limit for arsenic of 10 picograms. This sensitivity is, of course, retained in the HPLC-GFAA system for each separate analysis. Upon migration through the chromatographic column the arsenic compounds are separated and spread out into bands. Aliquots of 40 µL are withdrawn from the effluent. The 40 µL aliquots taken from the center of a band, where the arsenic concentration is highest, must each contain at least 10 picograms of arsenic. The detection limit of the HPLC-GFAA system is therefore strongly dependent on the degree of band spreading. Under optimized conditions ten nanograms of an arsenic compound placed on the column should produce discernible GFAA signals. The sensitivity of the HPLC-GFAA system can be increased by optimization of the chromatographic parameters, by using multiple injection - drying cycles before atomization or by pre-concentration of the arsenic compound on the top of the chromatographic column.

THE SPECIATION OF ARSENITE, ARSENATE, METHYLARSONIC ACID, DIMETHYLARSINIC ACID, ARSENOCHOLINE AND ARSENOBETAINE USING THE HPLC-HITACHI ZEEMAN GFAA SYSTEM

Arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, arsenocholine and arsenobetaine are arsenic compounds which might be present in natural water not polluted by human activities. Methods were developed for the separation and determination of these compounds by HPLC employing a Hitachi Zeeman graphite furnance atomic absorption spectrometer as an arsenic-specific detector. The HPLC-GFAA system will not only indicate the presence or absence of the arsenic compounds for which the separation methods were designed but will also show the presence of other arsenic compounds. The possibility that an unknown arsenic compound has the same retention time (volume) as one of the arsenic compounds named above does exist. However, chromatograms using several mobile phases and mass balances should reveal the presence of additional compounds.

Separation of inorganic arsenic, arsenocholine and arsenobetaine:

Arsenocholine, arsenobetaine and inorganic arsenic (arsenite and arsenate) were separated on a micro-particulate (10 µ) C-18 reverse phase column with the sodium salts of heptanesulfonic acid or dodecylbenzenesulfonic acid as counter ions for the arsonium salts (57, 58).

Arsenocholine, arsenobetaine and inorganic arsenic required mixtures of acetonitrile, water and counter ions as mobile phases, which are made 1.0 M with respect to acetic acid to suppress the dissociation of the carboxylic acid group in arsenobetaine and thus achieve greater retention on the reverse phase column. By varying the ratio acetonitrile/water in the eluent the retention of arsenocholine can be changed from total retention to co-elution with arsenobetaine (Fig. 6). Arsenobetaine cannot be totally retained with heptansulfonic acid as the counter ion. However, total retention on the top of the column was achieved with dodecylbenzenesulfonate. This procedure is useful for the pre-concentration of arsenobetaine from very dilute solutions. Arsenite and arsenate were not retained at all with either of the sulfonates on the C-18 reversed phase column. These inorganic

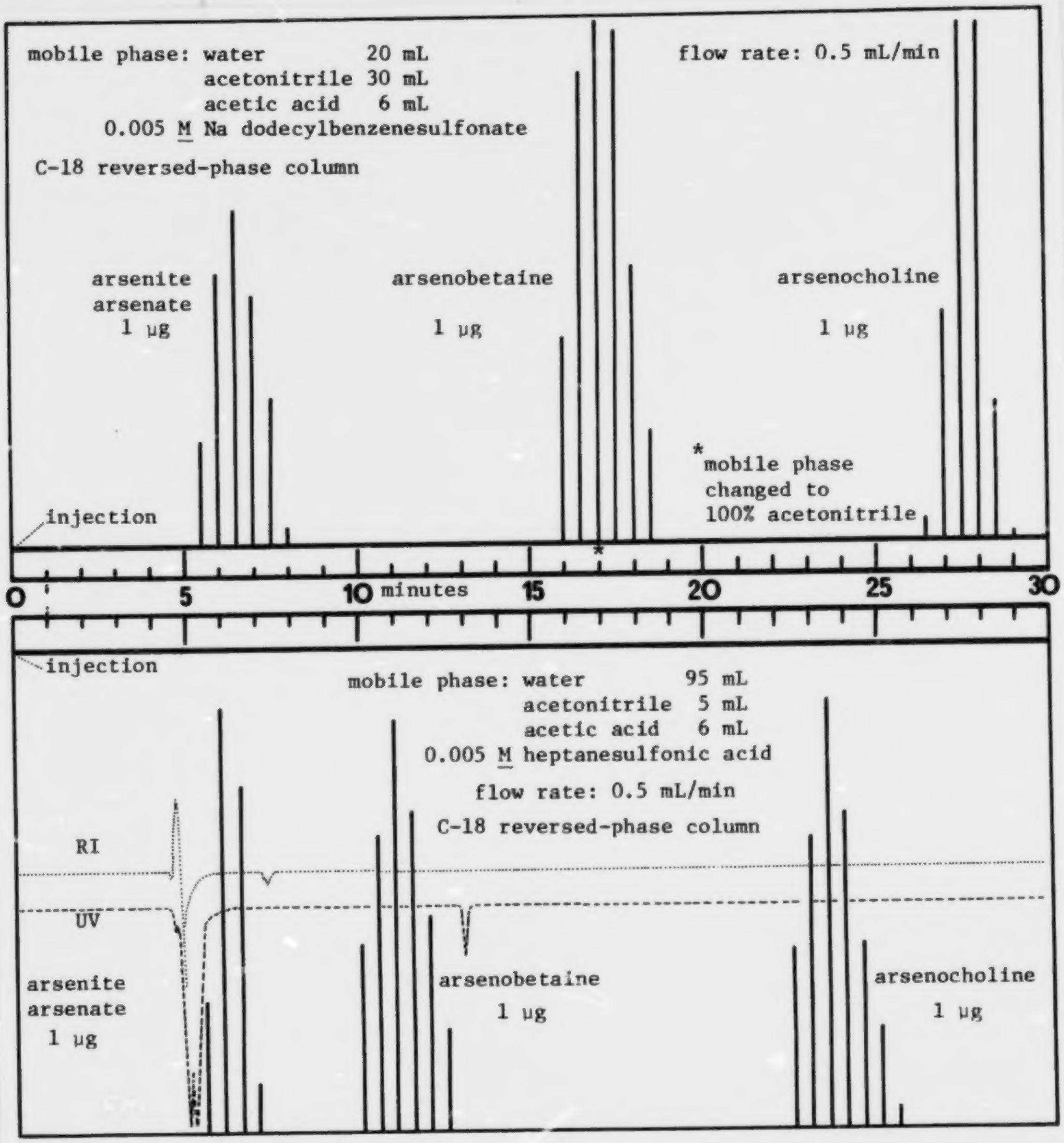

Figure 6. Separation of inorganic arsenic (arsenite and arsenate), arsenocholine and arsenobetaine by HPLC-GFAA on a C-18 reversed-phase column.

(From reference 57; redrawn with permission of Gordon and Breach Science Publishers) arsenic compounds eluted with the solvent front. Peaks caused by arsenic-free impurities in the solvent or the samples were recorded by the refractive index (RI) and UV detectors. These detectors were not sensitive enough to respond to the arsenic compounds. However, the GFAA detector has the required sensitivity to show the presence of the arsenic compounds (Fig. 6). No peaks other than those indicating the presence of arsenic compounds will appear in the GFAA chromatograms facilitating the quantitation and identification of arsenic compounds even in complex matrices which would produce a large number of RI and UV signals.

The separation and determination of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid: A C-18 reversed-phase column can be used to separate ionic species provided the ions are paired with a suitable lipophilic counter ion to produce a rather hydrophobic ion pair. Tetrabutylammonium phosphate (TBAP) at 0.005 *M* concentration in the appropriate mobile phase has been recommended as the reagent for such paired-ion chromatography. Arsenite and arsenate migrated almost with the solvent front through a C-18 reversed-phase column with water-methanol (95:5 v/v) at pH 7.3 as the eluent in the absence of TBAP but were completely separated in the presence of TBAP (0.005 *M*) with retention times of 19 min and 11 min for arsenate and arsenite, respectively (Fig. 7). When a mixture of arsenate, arsenite, dimethylarsinic acid (DMAA) and methylarsonic acid (MAA) was chromatographed with 0.005 *M* TBAP in pure water as eluent, which should increase the retention time of lipophilic ion pairs, the peaks caused by arsenite and DMAA overlapped. The MAA signals were located under these conditions between those belonging to arsenite and arsenate. TBAP does not cause sufficient retention and separation even with water as the mobile phase and has the additional disadvantage of containing phosphate. Phosphoric acid used to adjust the pH of the mobile phase and ammonium phosphates added as a source of counter ions depressed the arsenic signals (Fig. 8) Such interferences have been mentioned in the literature (60). Although phosphates have been recommended for use in HPLC because of their compatibility with the stainless steel columns, nitrates or perhaps organic anions should be used for work with arsenic compounds.

A much better separation was achieved with tetraheptylammonium nitrate (THAN). Utilizing water-methanol (75:25 v/v) of pH 7.6 saturated with THAN as the mobile phase all four arsenic compounds were satisfactorily separated (Fig. 9). Arsenite eluted almost with the solvent front, followed by DMAA, MAA and arsenate. The retention times of these compounds increase with increasing pK_a values of the arsenic acids. Arsenous acid ($pK_a = 10$) remains undissociated under these conditions, does not pair with the tetraheptylammonium cation, retains its polar character and is, therefore, not adsorbed onto the organic, reverse-phase column material. The weak acid DMAA ($pK_a 6.3$) owes its retention time of 10 min to the two methyl groups rather than to its presence in solution as the tetraheptylammonium dimethylarsinate ion pair. The moderately acidic MAA ($pK_a 3.6$) is very likely combined with the ammonium ion and adsorbed by the column material as the tetraheptylammonium methylarsonate resulting in a retention time of 23 min. Arsenic acid with a pK_a value of 2.2, the most acidic compound among the four arsenic derivatives investigated, is strongly retained on the column head probably as the ion

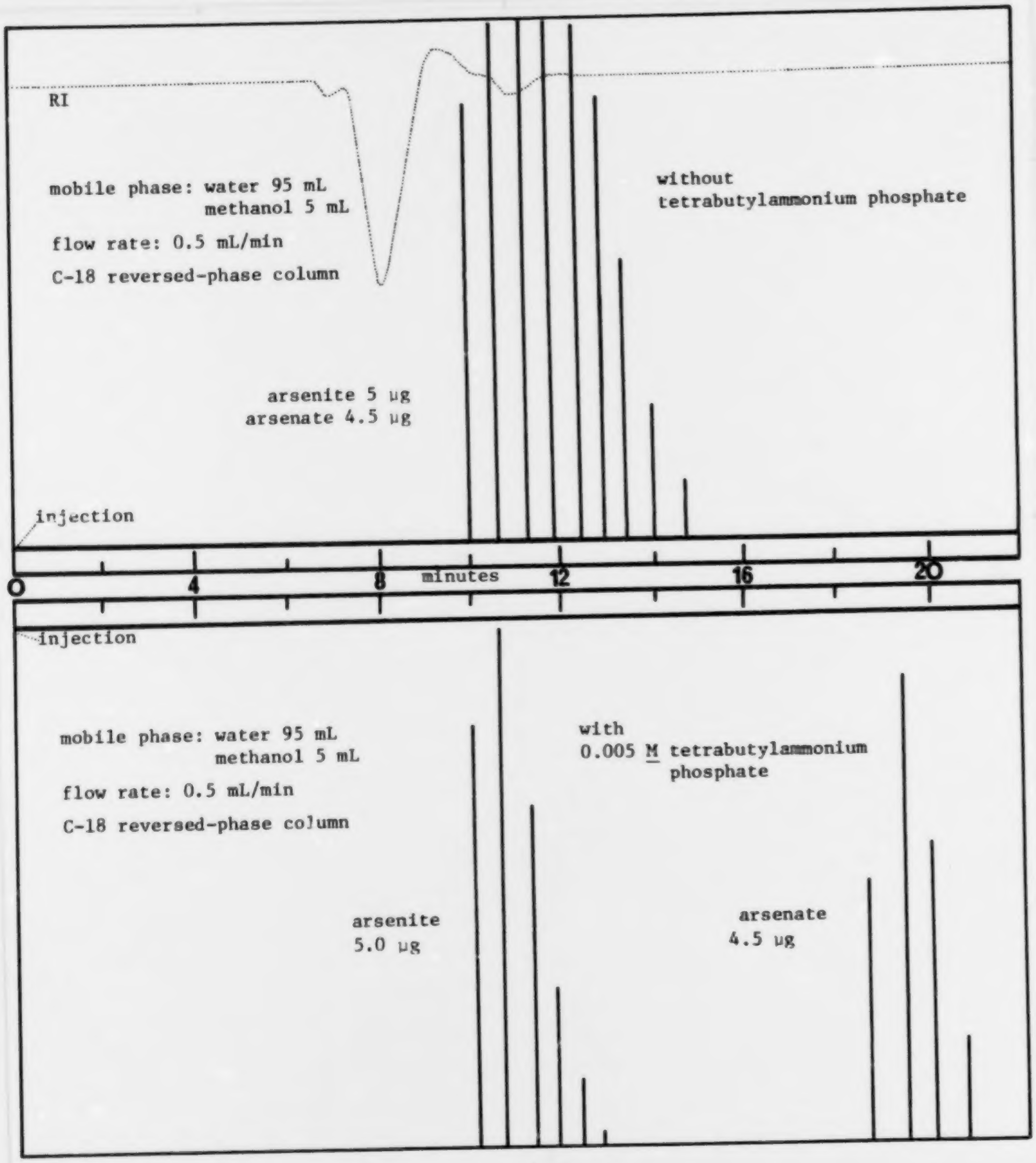

Figure 7. Separation of arsenite and arsenate by HPLC-GFAA on a C-18 reversed-phase column in the absence and presence of tetrabutylammonium phosphate.

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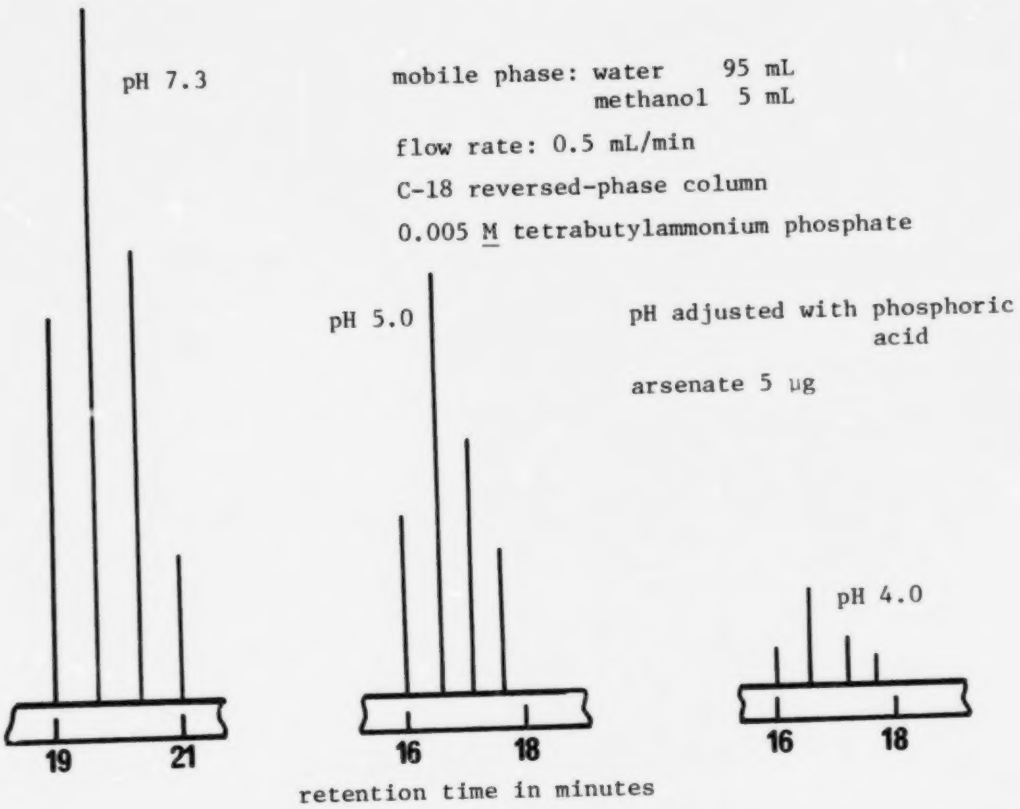

Figure 8. The influence of phosphate on the GFAA arsenic signals.

pair $(R_4N)_2^+ HAsO_4^{--}$. The mobile phase had to be changed to pure methanol to elute the arsenate.

The GFAA signals produced by the arsenic compounds eluting from the columns can be quantitated either by calculating the area under the curves obtained by connecting the maxima of the GFAA signals or by summing the heights of the signals corresponding to a particular compound. A comparison of the two methods to convert the GFAA signal clusters corresponding to a particular arsenic compound to numerical values to be plotted against the amounts of arsenic, proved that the summation of peak heights produces the same results as the determination of the areas under the signal clusters (58). Examples of calibration curves are given in Figs. 10 and 11.

The sensitivity and general response of the GFAA instruments is rather strongly dependent on the characteristics of the graphite cups and other parts of the furnace system. The cups, for instance, tend to deteriorate with use and reduce the sensitivity. A new cup does not necessarily produce

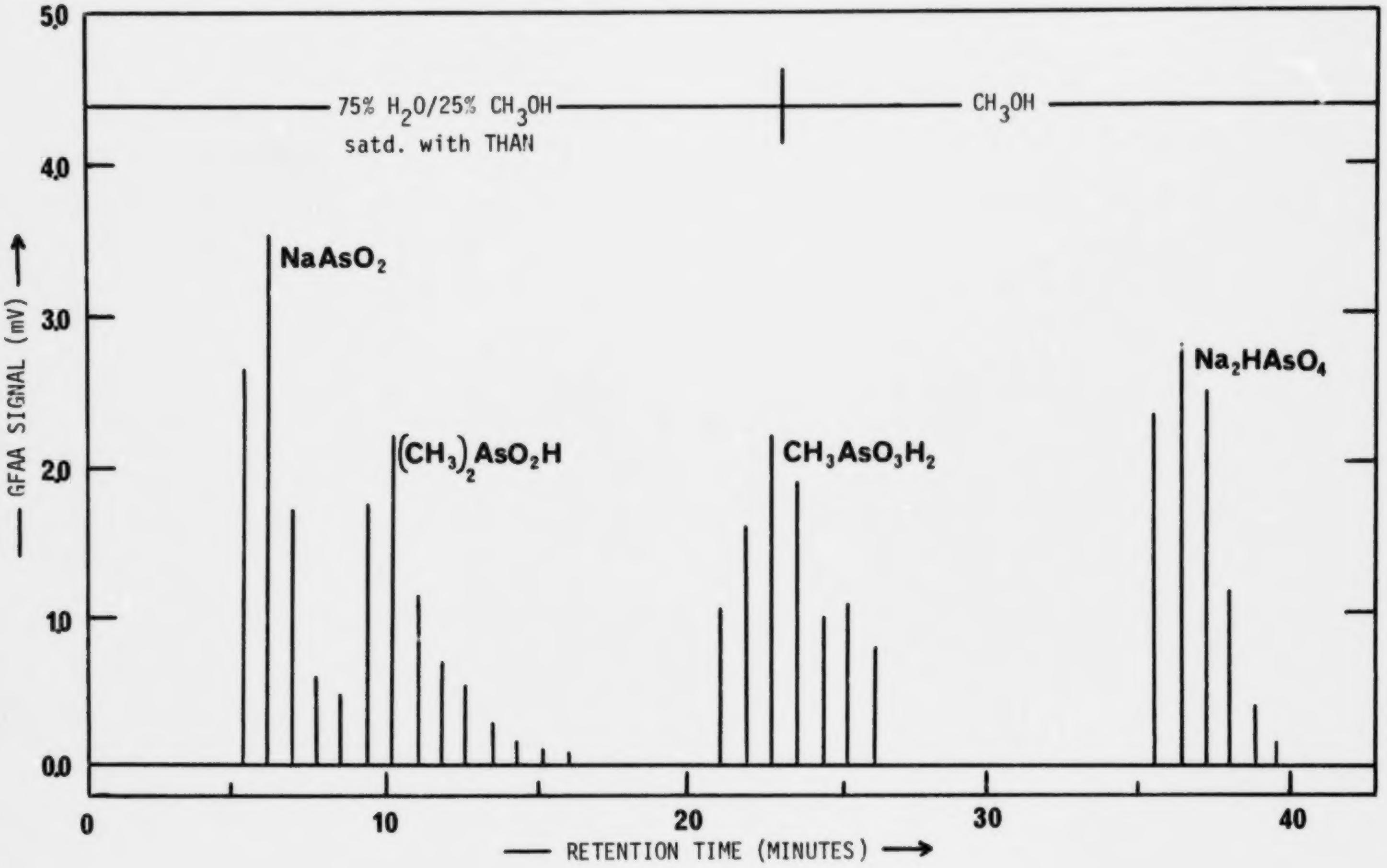

Figure 9. The separation of arsenite, arsenate, dimethylarsinic acid and methylarsonic acid by HPLC-GFAA on a reversed-phase column in the presence of tetraheptylammonium nitrate.

(From reference 58; used with permission of Elsevier Scientific Publishing Company)

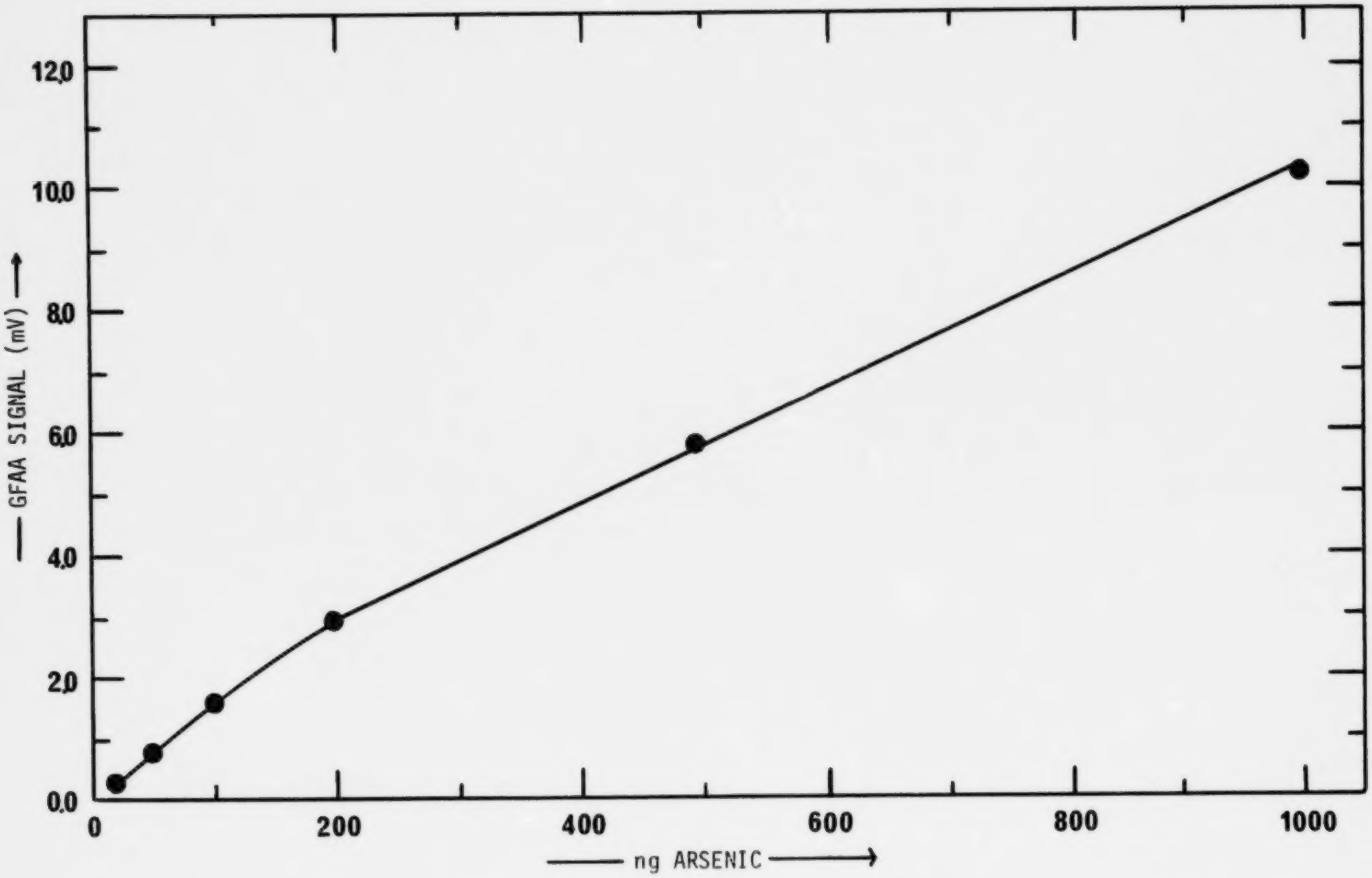

Figure 10. HPLC-GFAA calibration curve for sodium arsenite.

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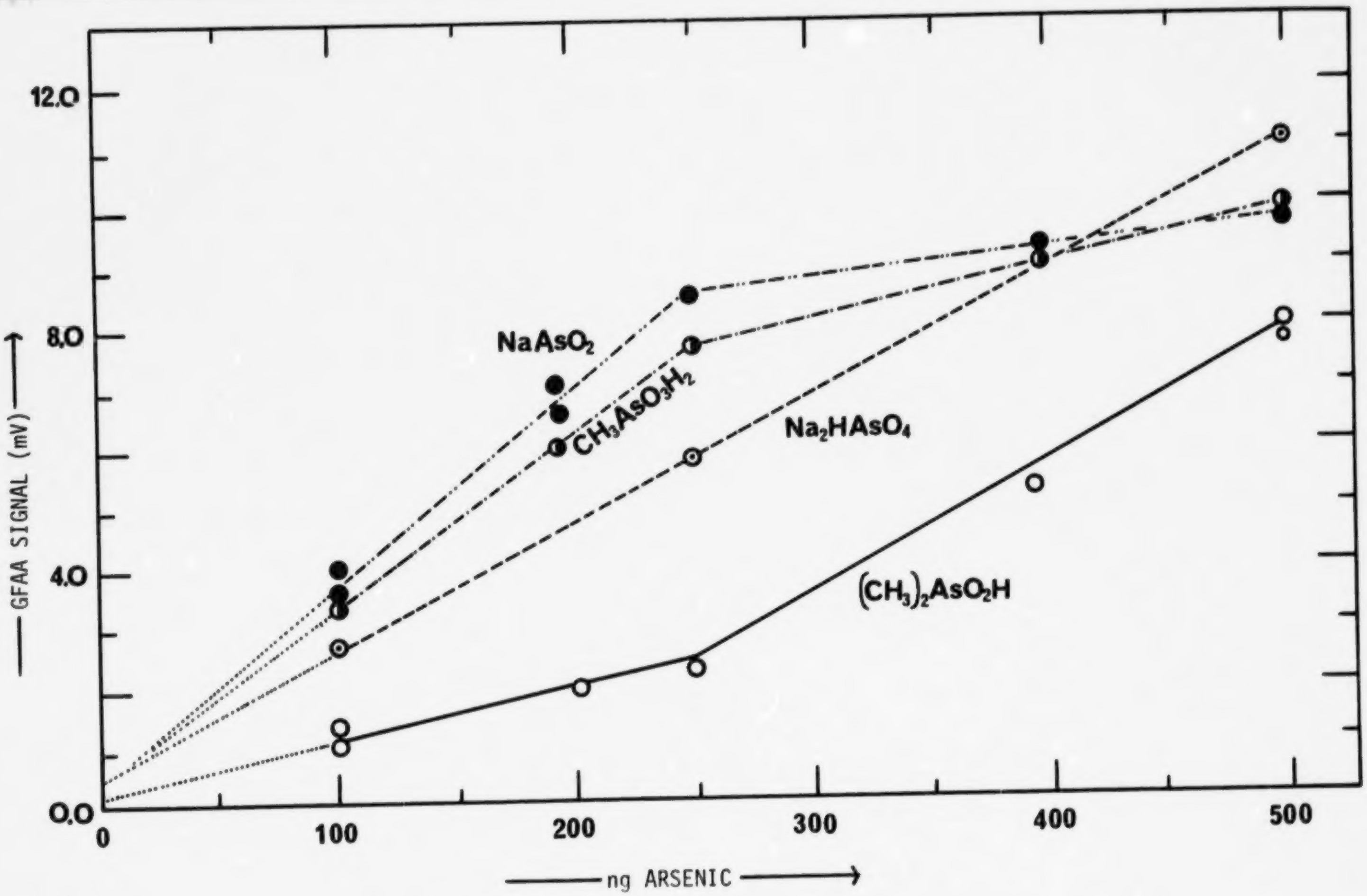

Figure 11. HPLC-GFAA calibration curves for sodium arsenite, dimethylarsinic acid, methylarsonic acid and disodium hydrogen arsenate.

(From reference 58; used with permission of Elsevier Scientfic Publishing Company) the same response as one subjected to a number of analysis cycles. This behavior necessitates the frequent use of standards. The Hitachi Zeeman GFAA with a sensitivity of 10 picograms of arsenic serving as a detector for the effluent from a C-18 reverse phase column is capable of detecting several nanograms of arsenic under ideal conditions (Fig. 10) and approximately 10 nanograms of sodium arsenite in the presence of DMAA. The determinations are accurate with ±5-10 percent and are intrinsically background-corrected. The causes of the changes in the slopes of the calibration curves are not clear yet and need to be investigated further.

Various arsenic compounds produce different GFAA responses for the same amount of arsenic (58). As much as a two-fold difference in intrinsic GFAA sensitivity was observed with DMAA and MAA as well as between their sodium salts. The less volatile species give a more intense signal than the more volatile compounds. On the basis of these results quantitative information for unknown arsenic compounds obtained with the HPLC-GFAA system must be regarded to be qualitative, until the compounds are identified and calibration curves constructed with pure samples.

The HPLC-GFAA system has been shown to be applicable to the qualitative and quantitative determination of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid in the ppb range with detection limits approaching 5 ppb under favorable conditions. The GFAA detector responds only to a specific element and is better suited for trace element compound determination than UV detectors. Development of column materials will lead to better separations. Recently the use of a proprietary special anion-exchange column for the separation of arsenic compounds (61) and an automated system for the analysis of arsenite, arsenate, methylarsonic acid, 4-aminophenylarsonic acid and dimethylarsinic acid based on the separation with a Dionex anion exchange column (62) was reported.

DIFFERENTIAL PULSE POLAROGRAPHIC DETERMINATION OF ARSENATE AND ARSENITE

Arsenite is reducible at the dropping mercury electrode. A very detailed review of the literature about the polarographic behavior of arsenite appeared in 1969 (63). In hydrochloric, perchloric, sulfuric or acetic acid media arsenite is reduced polarographically to elemental arsenic and then to arsine. The best supporting electrolyte among those thus far investigated is hydrochloric acid. In 1 M hydrochloric acid the detection limit is 0.3 ppb with a linear response up to 60 ppm (37).

Arsenate is polarographically inactive under the conditions used for the polarographic determination of arsenite. However, arsenate produced polarographic waves in 11.5 M hydrochloric acid (64) and in 2 M perchloric acid in the presence of pyrogallol (65). Arsenate gave three polarographic waves in pyrogallol, the heights of which increased linearly with concentration. Solutions with concentrations lower than 37 ppm arsenic were not investigated (65). Pyrogallol is difficult to handle, is easily oxidized by air and may pose health hazards. The use of a 11.5 M hydrochloric acid medium for routine analysis is untenable. Several other polyhydroxy

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compounds were investigated as reagents to make arsenate polarographically active and develop a method to determine arsenate and arsenite simultaneously at ppb levels.

Differential Pulse Polarographic(DDP) Determination of Arsenate in the Absence of Arsenite

Pyrocatechol, pyrogallol, 1,2-ethanediol, 1,2-propanediol, 3-chloro-1,2-propanediol, 1,2-butanediol, 2,3-butanediol, D-ribose, sucrose, fructose and D-mannitol were investigated. All of these polyhydroxy compounds made arsenate polarographically active. The peak heights of the DPP curves for the reduction of 10 ppm As(arsenate) in solution 2.0 M with respect to perchloric acid and 0.5 M with respect to the polyhydroxy compound are listed in Table 2. An example of the DDP curve produced by arsenate in the presence of mannitol is given in Fig. 12. Under these conditions D-mannitol produced the largest signal.

TABLE 2. PEAK HEIGHTS OF THE DIFFERENTIAL PULSE POLAROGRAPHIC CURVES AT -0.55 V OBTAINED WITH 2.0 M AQUEOUS PERCHLORIC ACID SOLUTIONS OF 100 ppm As(ARSENATE) IN THE PRESENCE OF VARIOUS POLYHYDROXY COMPOUNDS AT 0.5 M CONENCTRATION

Polyhydroxy Compound	Peak Height at -0.55 V in mm
3-Chloro-1,2-propanediol	45.7
Pyrocatechol	68.6
Pyrogallol	71.1
1,2-Ethanediol	93.9
1,2-Propanediol	132.1
1,2-Butanediol	142.2
D-Ribose	170.2
Sucrose	172.7
Fructose	182.8
2,3-Butanediol	185.4
D-Mannitol	203.2

The heights of the reduction waves are influenced by the concentration of mannitol. As shown in Fig. 13 these heights become independent of the mannitol concentration after three grams of mannitol had been added to 50 mL of the sample. To make certain that an excess of mannitol is always present

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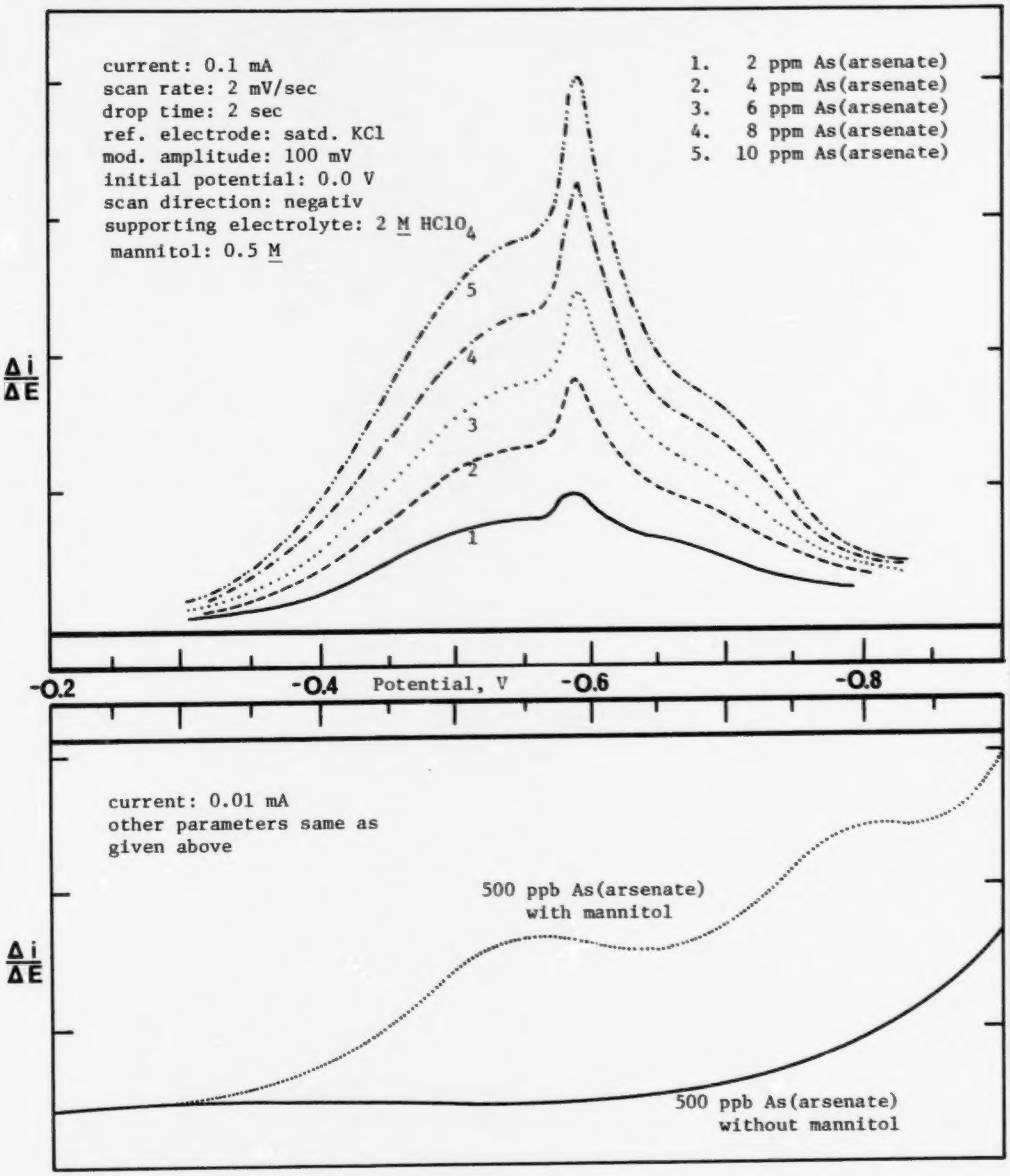

Figure 12. Differential pulse polarograms of arsenate solutions two molar with respect to perchloric acid in the presence of D-mannitol.

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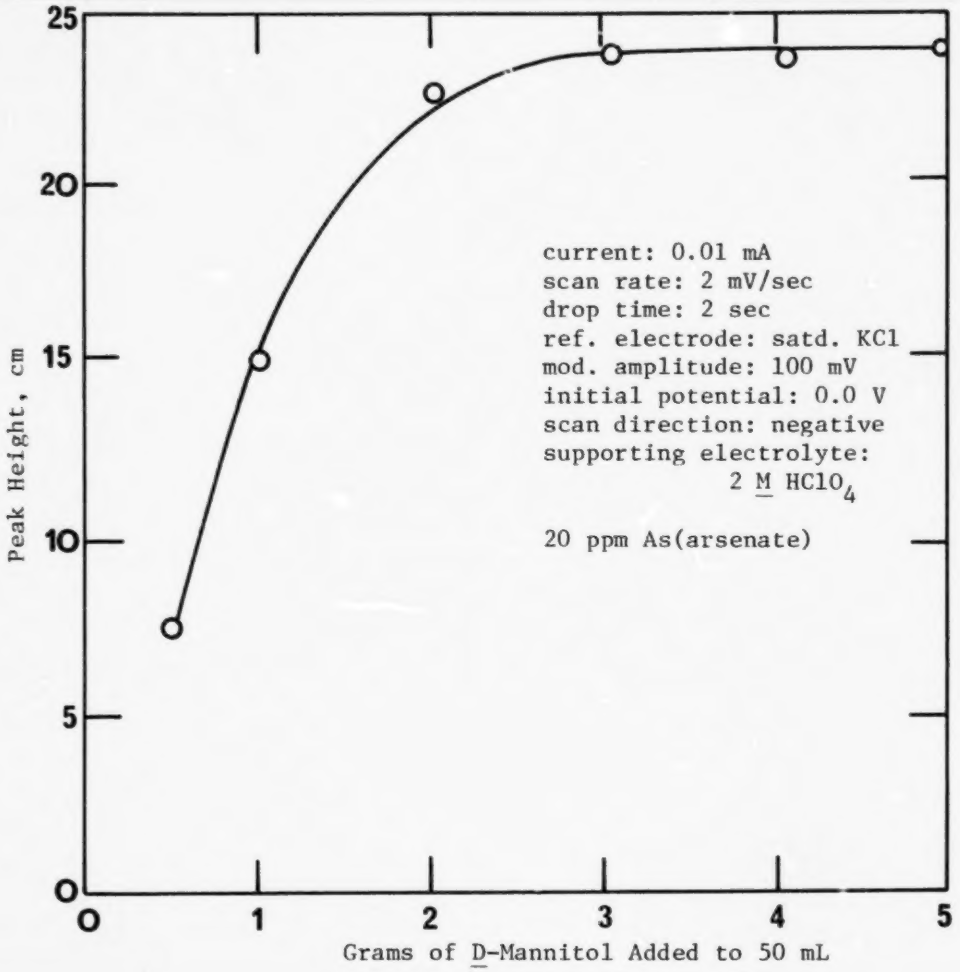

Figure 13. The dependence of the height of the differential pulse polarographic curve of arsenate at -0.55 V on the concentration of D-mannitol.

in the solutions to be polarographed and thus assuring the independence of the heights from the mannitol concentration, all further experiments were carried out with solutions prepared by addition of 4.5 g of mannitol to 50 mL of the acidified sample. These solutions are approximately 0.5 M with respect to mannitol.

Sulfuric, hydrochloric, acetic, phosphoric and perchloric acid were investigated as supporting electrolytes in the DPP determination of arsenate in solutions containing mannitol at 0.5 M concentration. The solutions were all 2.0 M with respect to the acid to be investigated. The results of these

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experiments are summarized in Table 3. Perchloric acid produced the largest signal among the acids tested and was used in all further experiments.

TABLE 3. HEIGHTS OF THE FIRST DIFFERENTIAL PULSE POLAROGRAPHIC MAXIMUM IN THE REDUCTION OF 9.5 ppm As(ARSENATE) IN AQUEOUS ACIDIC MEDIA IN THE PRESENCE OF *D*-MANNITOL AT 0.5 MOLAR CONCENTRATION

Acid	Peak Height in mm	Potential of Maximum
Sulfuric	7.6	-0.45 V
Hydrochloric	15.2	-0.55 V
Acetic	17.8	-0.90 V
Phosphoric	20.3	-0.45 V
Perchloric	22.8	-0.44 V

*All solutions were 2.0 *M* with respect to the acid.

The reduction waves and the current maximum shifted anodically and the heights of the shoulders on the anodic and cathodic side of the current maximum increased with increasing perchloric acid concentration (Fig. 14). For example, an increase of the perchloric acid concentration from 0.94 *M* to 5.8 *M* shifted the first reduction wave from -0.57 V to -0.50 V and doubled the peak height. Because of the difficulty of adjusting water samples to a perchloric acid concentration of five molar, two molar perchloric acid was chosen as a compromise providing satisfactory sensitivity and relative ease of sample preparation.

The DPP curve of arsenate in an aqueous perchloric acid/mannitol solution is characterized by maxima at -0.55 V and -0.75 V (Fig. 12). Above an As(arsenate) concentration of 500 ppb a current maximum appears at -0.59 V which increases in intensity with increasing concentration. The peak at -0.55 V merges at As(arsenate) concentrations of approximately 5 ppm into the current maximum and becomes a shoulder. Another rather low intensity peak between -0.7 V and -0.8 V might be obscured at low arsenate concentrations by the solvent breakdown and at high arsenate concentrations by the current maximum. Since arsenite is reduced in 2.0 *M* perchloric acid in the presence of mannitol at -0.34 V, it is likely that the first arsenate reduction wave at -0.55 V is caused by the reduction As(V) → As(0) followed by the conversion of As(0) to AsH_3 at approximately -0.7 V. Experiments which would verify these postulated steps have not been carried out yet.

The differential pulse polarographic maximum or shoulder at -0.55 V generated by arsenate in 2.0 *M* perchloric acid in the presence of mannitol is suitable for the quantitative determination of arsenate. The current maximum could perhaps be used for the determination of arsenate at

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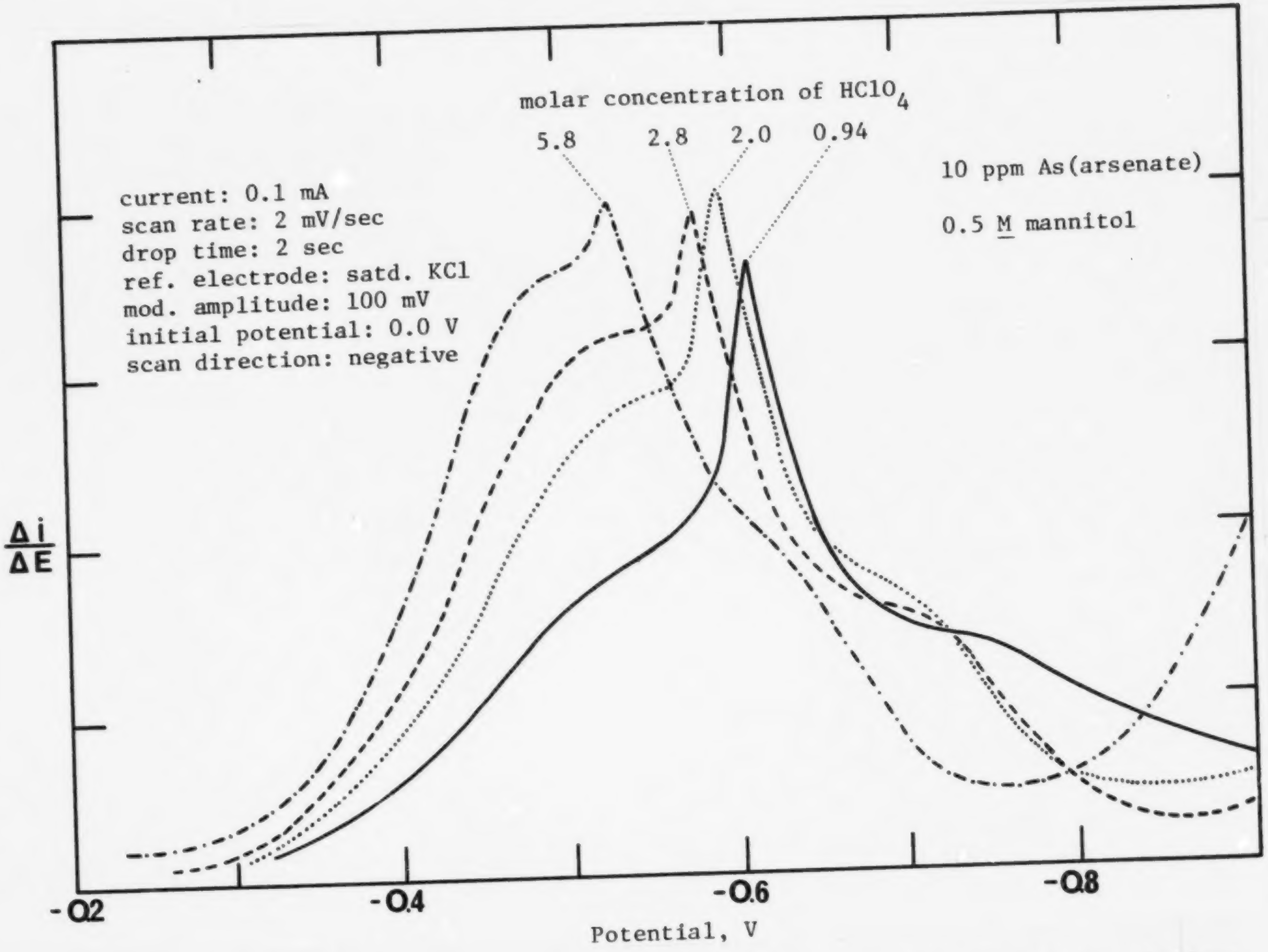


Figure 14. Differential pulse polarographic curves for 10 ppm As(arsenate) in solutions 0.5 M with respect to D-mannitol at various perchloric acid concentrations.

concentrations above 1 ppm As(arsenate).

Calibration curves for the determination of arsenate in the absence of arsenite were obtained by addition of 20 μL aliquots of an arsenate solution of appropriate concentration to 50 mL of sample solution in the polarographic cell. After each addition the differential pulse polarogram was recorded. An example of a calibration curve using the -0.55 V maximum (shoulder), is given in Fig. 15. By proper adjustment of the current, linear calibration curves were obtained for As(arsenate) concentrations ranging from 20 ppb to 900 ppb at 2 μA, 1 ppm to 18 ppm at 50 μA and 20 ppm to 160 ppm at 0.5 mA.

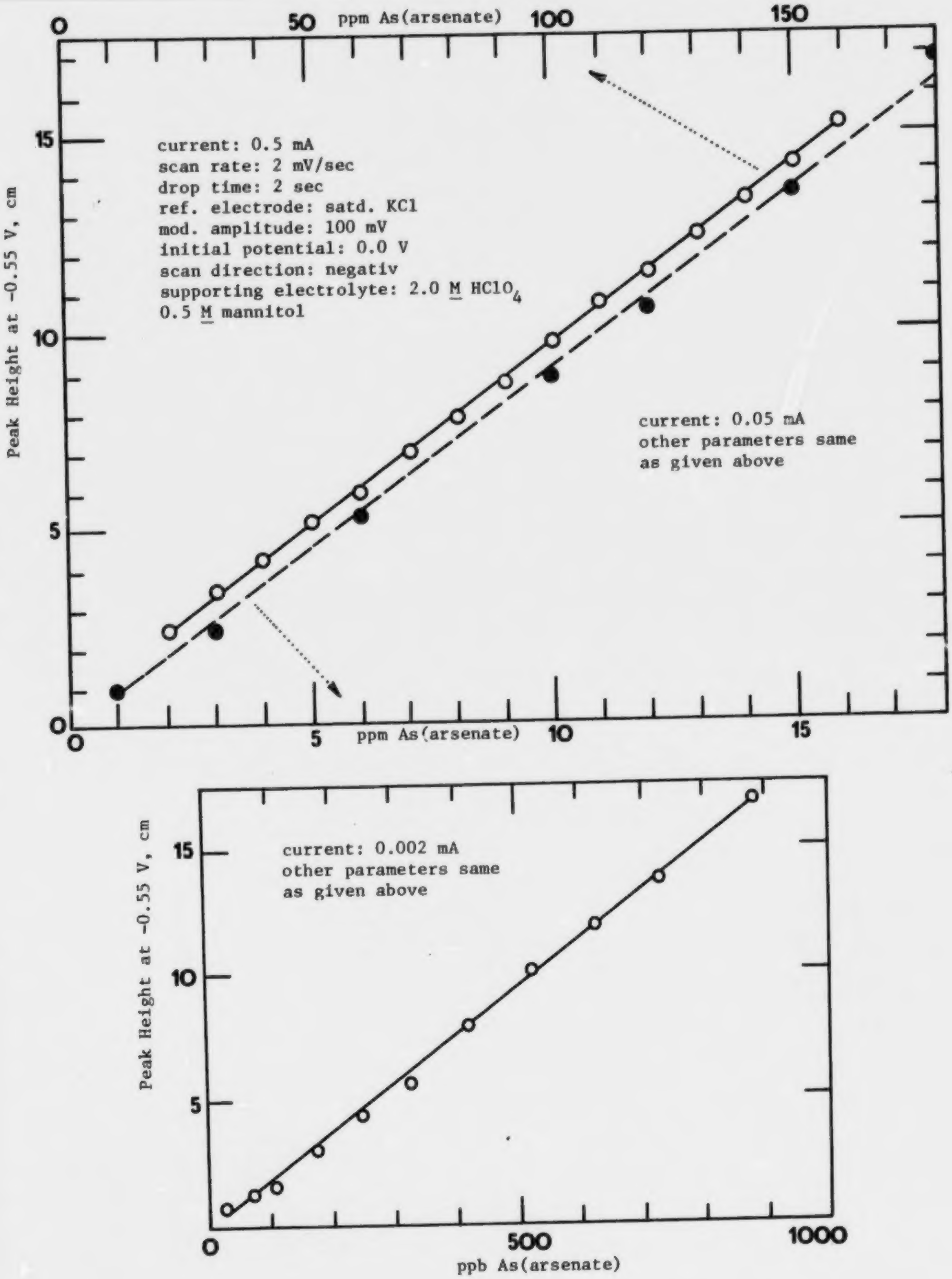
Differential Pulse Polarographic Determination of Arsenite in the Absence of Arsenate

Arsenite does not require mannitol for its reduction at the dropping mercury electrode. In 2.0 molar perchloric acid solution arsenite is reduced to As(0) at -0.425 V. As(0) is converted to arsine at approximately -0.75 V. A current maximum appears at an As(arsenite) concentration of approximately 300 ppb. The addition of mannitol to 2.0 M perchloric acid solutions of arsenite shifts the As(III) → As(0) reduction anodically to -0.34 V, but does not affect the position of the current maximum and the wave at -0.75 V (Fig. 16). The heights of all the maxima are increased. The location of the maxima is dependent on the perchloric acid concentration. The As(III) → As(0) peak moves from -0.49 V at 0.7 M to -0.31 V at 4.7 M perchloric acid concentration.

An example of a calibration curve for the determination of arsenite in the range 0.2 to 2.4 ppm As(arsenite) in the presence of mannitol is given in Fig. 17.

Differential Pulse Polarographic Determination of Arsenate and Arsenite in Solutions Containing Both of these Arsenic Compounds

When arsenate and arsenite are present in the same solution, differential pulse polarography employing 2.0 M aqueous $HClO_4$ as supporting electrolyte, which is 0.5 M with respect to D-mannitol, will produce polarograms with maxima at -0.34 V [As(III) → As(0)], -0.55 V (arsenate reduction), -0.59 V (current maximum) and -0.75 V [As(0) → As(-III)] (Fig. 18). The arsenite reduction peak at -0.34 V [As(III) → As(0)] is well separated from the other maxima and can be used to quantitatively determine arsenite using the standard addition technique. The maximum at -0.55 V caused by the reduction of arsenate can be used for the determination of arsenate with some confidence only when the concentration of As(arsenite) is between 100 ppb and approximately 500 ppb, the current is not lower than 2 μA and the arsenate concentration is equal to or greater than the arsenite concentration. At As(arsenite) and As(arsenate) concentrations lower than 100 ppb (Fig 19) the polarograms must be obtained with a current of 0.5 μA. Although the current maximum at -0.59 V does not appear at these low concentrations, the arsenate reduction peaks occur as shoulders on the anodic side of the steep solvent breakdown signals. The shoulders can be used to ascertain the presence of arsenate and to semi-quantitatively determine arsenate. At an As(arsenite) concentration of 100 ppb and As(arsenate)



current: 0.5 mA
scan rate: 2 mV/sec
drop time: 2 sec
ref. electrode: satd. KCl
mod. amplitude: 100 mV
initial potential: 0.0 V
scan direction: negativ
supporting electrolyte: 2.0 M $HClO_4$
0.5 M mannitol

current: 0.05 mA
other parameters same
as given above

current: 0.002 mA
other parameters same
as given above

Figure 15. Calibration curves for the determination of arsenate by differential pulse polarography in aqueous 2.0 M perchloric acid in the presence of D-mannitol.

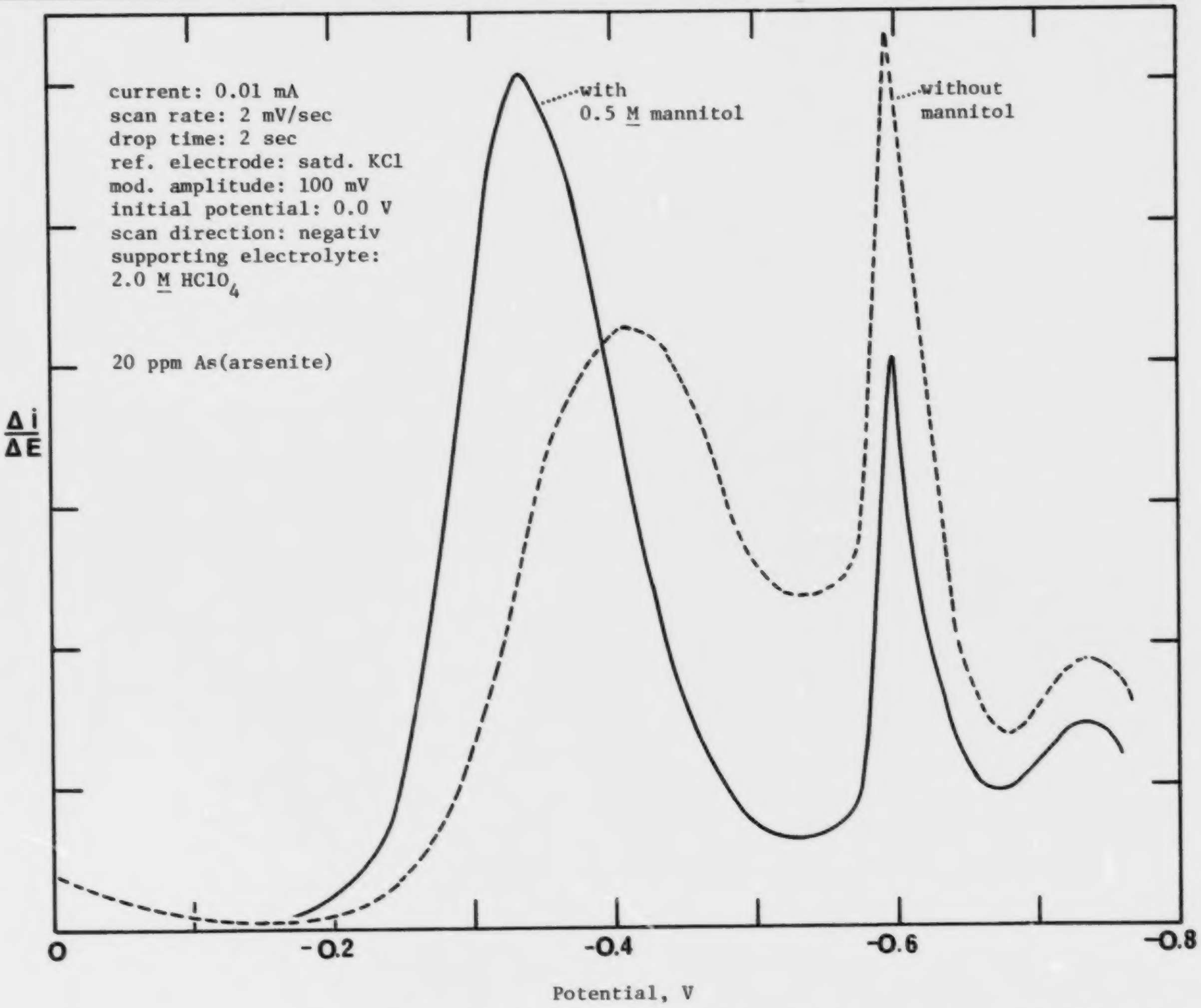


Figure 16. Differential pulse polarograms for arsenite in 2.0 M aqueous perchloric acid in the presence and absence of D-mannitol.

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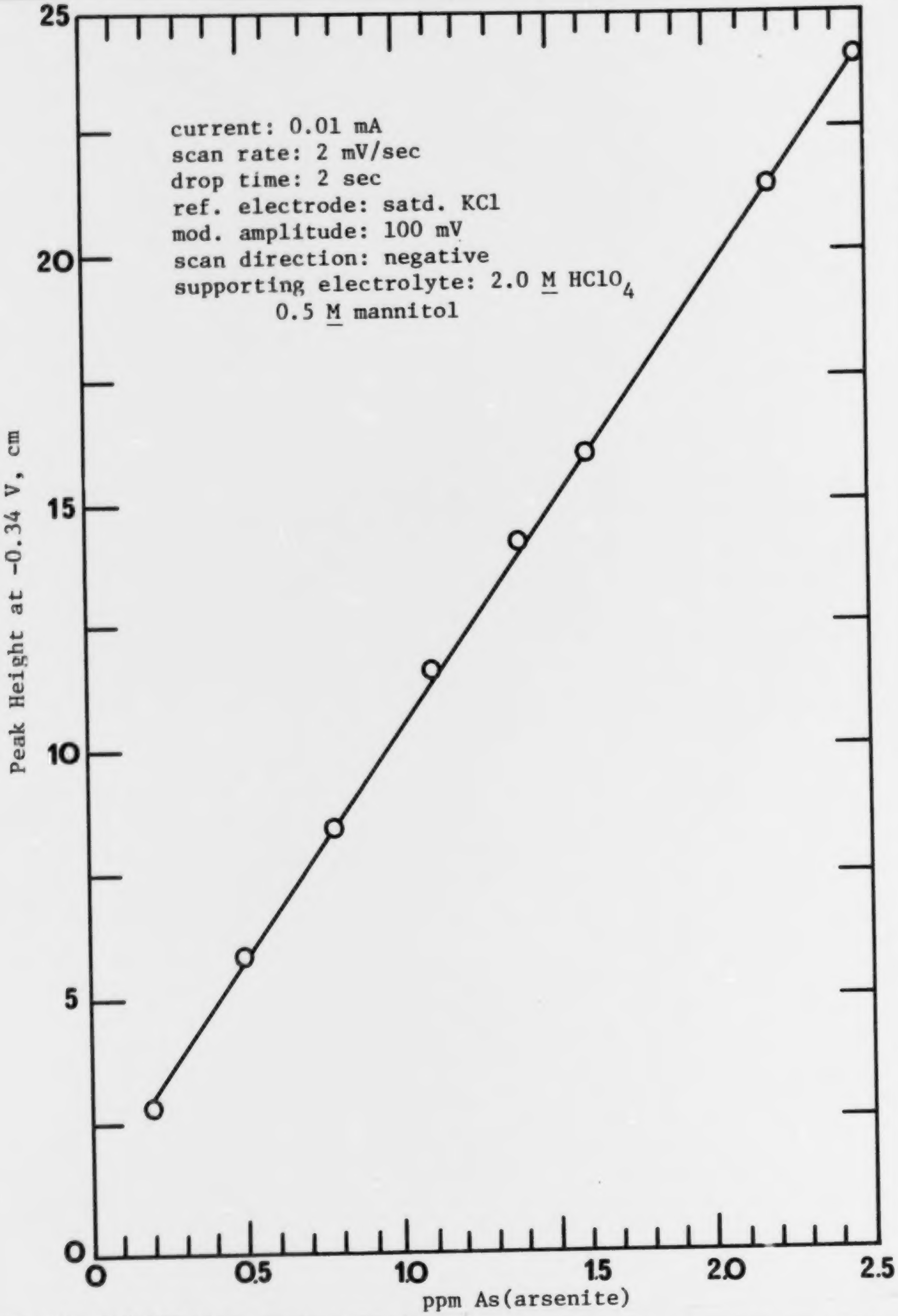

Figure 17. Calibration curve for the determination of arsenite by differential pulse polarography in aqueous 2.0 M $HClO_4$ in the presence of D-mannitol.

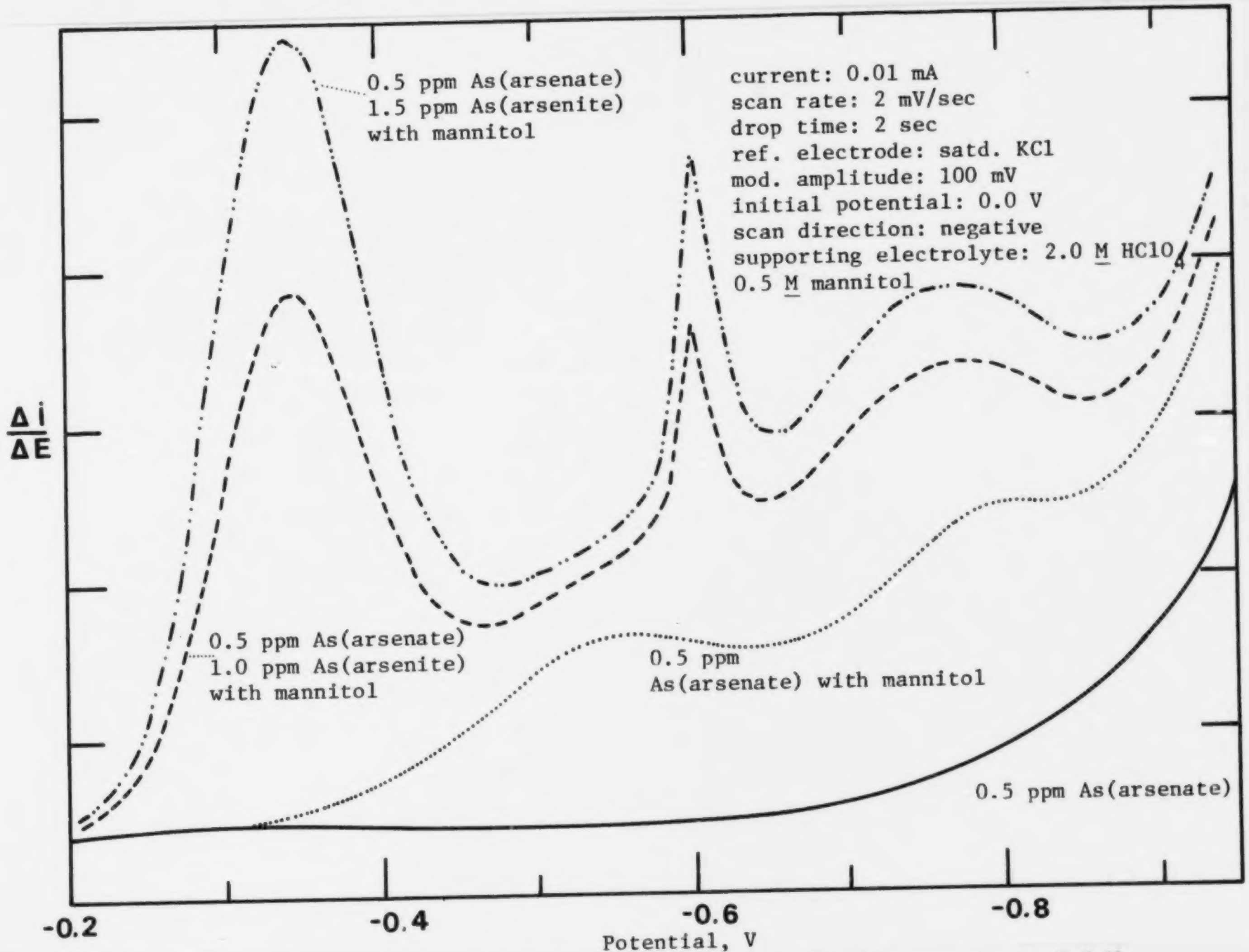

Figure 18. Differential pulse polarograms of mixtures of arsenite and arsenate in 2.0 M perchloric acid in the presence of D-mannitol.

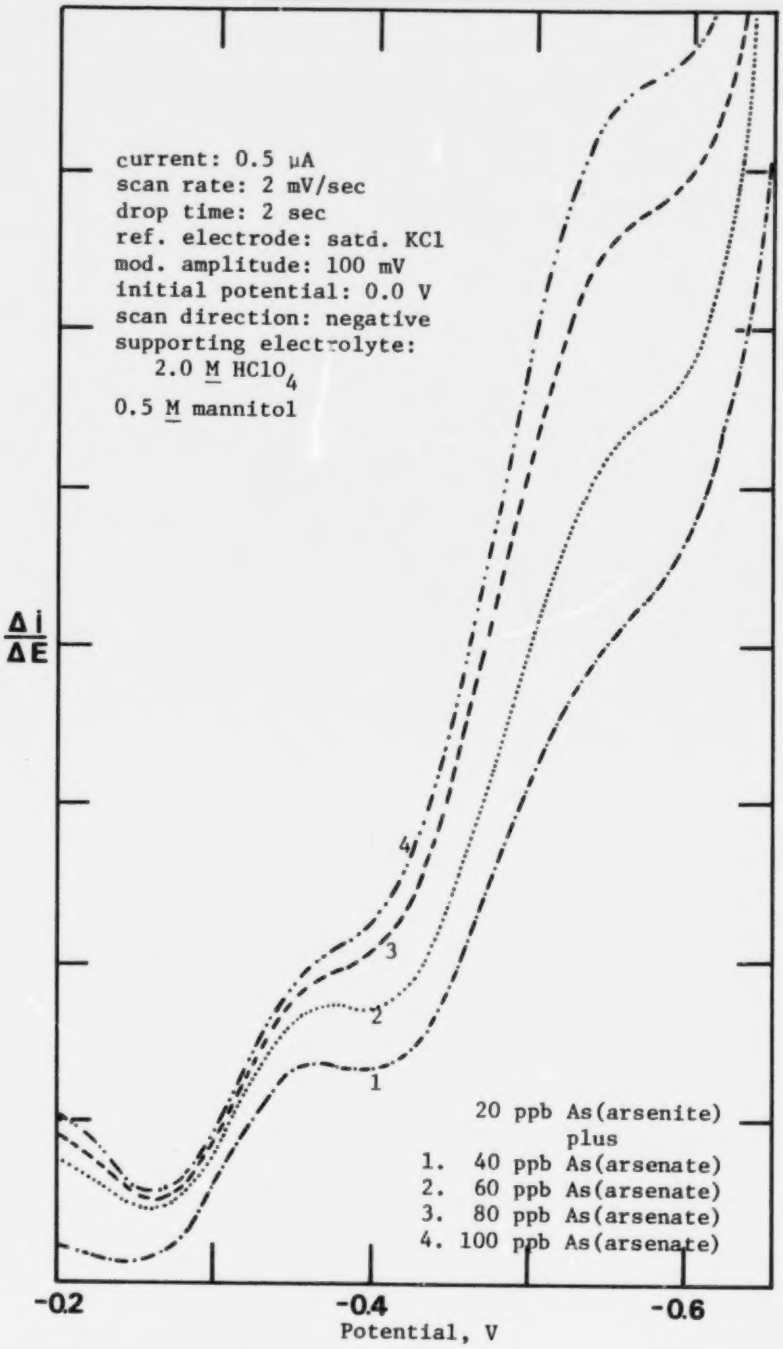

Figure 19. Differential pulse polarograms of solutions containing 20 ppb As(arsenite) and 40 to 100 ppb As(arsenate) in 2.0 M perchloric acid in the presence of D-mannitol.

concentrations in the range 200 to 300 ppb the polarogram can be obtained at 2 μA. Under these conditions the solvent breakdown curve is shifted cathodically, a current maximum is not present, and arsenate, therefore, may be determined by measuring the peak height of the -0.55 V maxima.

At As(arsenite) concentrations of 500 ppb with a current setting of 10 μA the solvent breakdown curve rises from the baseline beyond -0.8 V and, therefore, does not interfere with the -0.55 V maxima. However, at As(arsenite) concentrations of 500 ppb the current maximum at -0.59 V appears. The arsenate reduction peaks at -0.55 V are not resolved from the current maxima and appear as shoulders which make the quantitative determination of arsenate difficult.

At a concentration higher than approximately 0.5 ppm As(arsenate plus arsenite) the current maximum at -0.59 V, which is produced by arsenate as well as arsenite, could perhaps be used to determine total inorganic arsenic. Additional experiments are needed to clarify this issue.

Oxidation of Arsenite Followed by Polarography of Arsenate

The difficulties encountered in the direct determination of arsenate in the presence of arsenite can be overcome by quantitatively oxidizing arsenite to arsenate and determining the total inorganic arsenic as arsenate by differential pulse polarography with D-mannitol as reagent. As reported in the literature, the oxidation of arsenite to arsenate was achieved by addition of an approximately 200-fold molar excess of cerium(IV) ammonium nitrate in two equal proportions (66). An example of a polarogram of arsenate obtained by cerium(IV) oxidation of arsenite is presented in Fig. 20. The arsenite polarographic wave (curve 1) disappears completely upon oxidation of the arsenite. The arsenate-containing solution does not produce a polarogram in the absence of mannitol (curve 2). After addition of mannitol the arsenate reduction peak at -0.55 V can be used to determine the concentration of arsenate (curve 3).

The amount of cerium(IV) required for complete oxidation of 2×10^{-5} moles of arsenite was ascertained by successive addition of 40 μL of a 0.58 M cerium(IV) ammonium nitrate solution to the solution in the polarographic cell. After each addition the polarogram was recorded and the peak height at -0.34 V measured. A plot of the peak height versus the amount of cerium(IV) added (Fig. 21) shows that at least a 30-fold molar excess is required to completely oxidize arsenite to arsenate. To ascertain complete oxidation of arsenite, a 200-fold excess of cerium(IV) was employed in all experiments as recommended in the literature (66).

The excess cerium(IV) must be reduced to cerium(III) to avoid interferences in the polarographic determination of arsenate. It has been reported in the literature that the mercury on the bottom of the cell causes this reduction (66). However, erratic polarograms were obtained several times, which were caused by cerium(IV) still present in the solution.

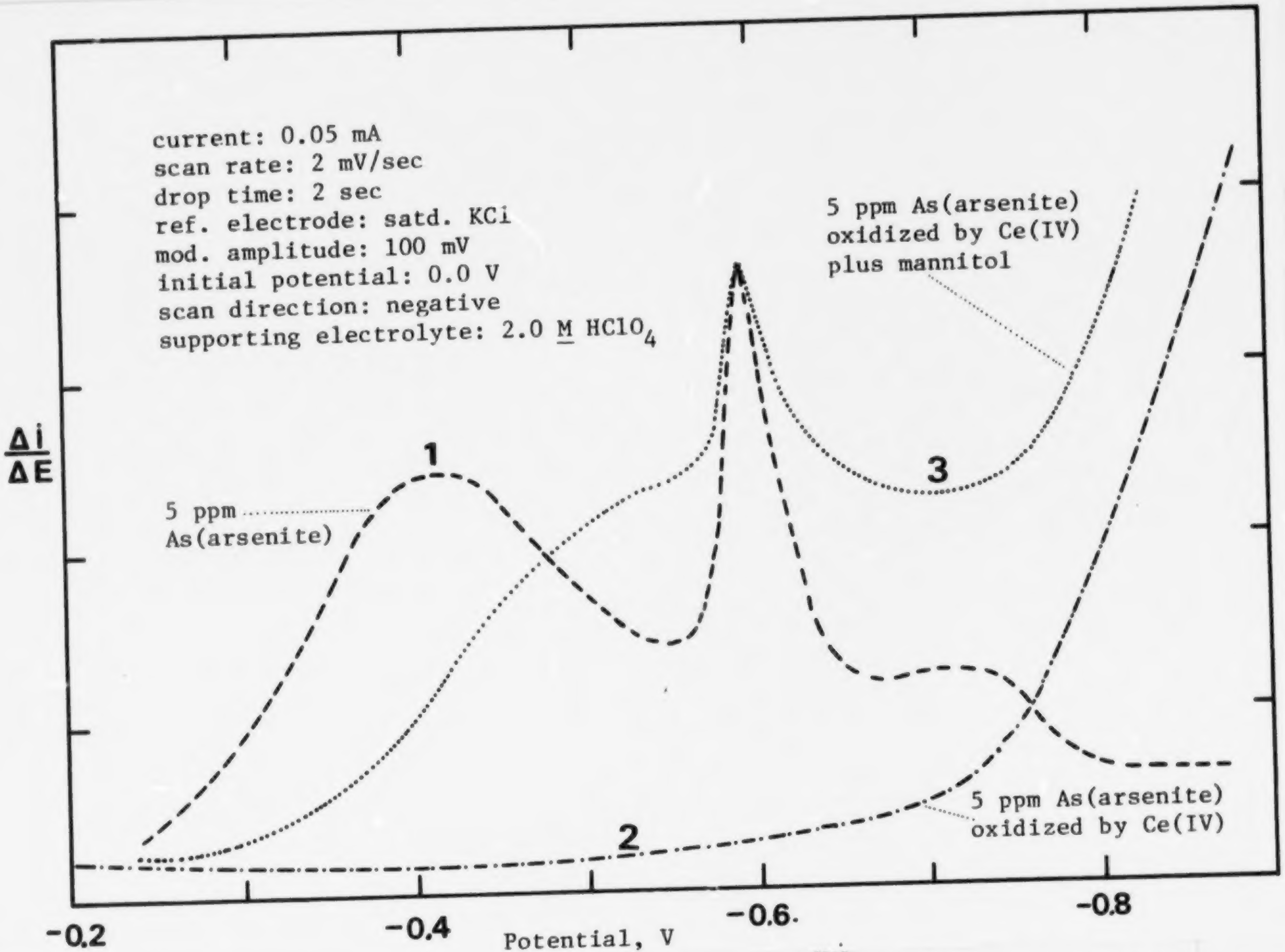

Figure 20. Differential pulse polarograms of arsenite, and of arsenate obtained by Ce(IV) oxidation of arsenite.

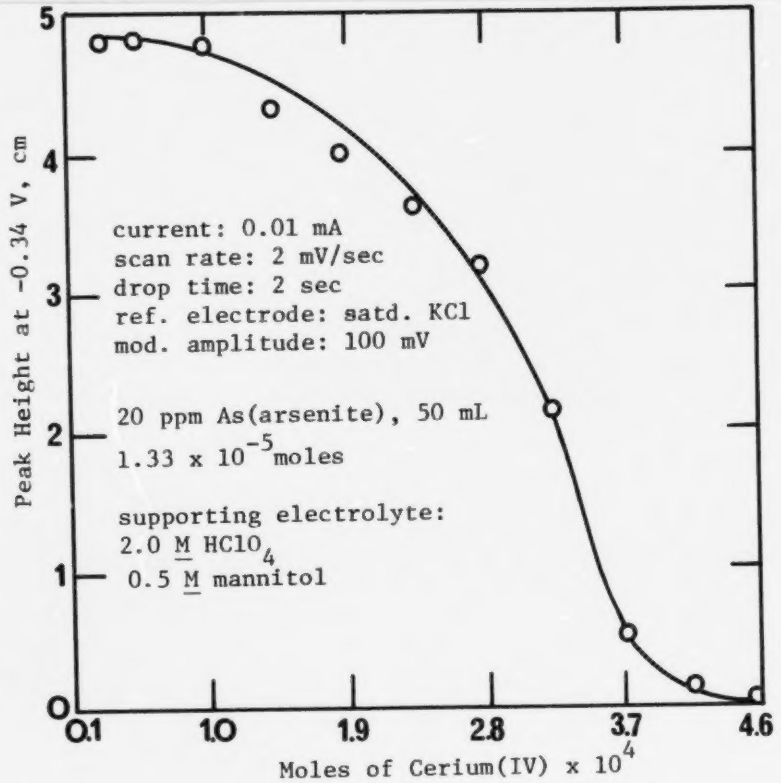

Figure 21. The dependence of the peak height of the differential pulse polarographic maximum at -0.34 V (arsenite reduction) as a function of the amount of cerium(IV) ammonium nitrate added.

The addition of approximately 0.35 g of hydroxylamine hydrochloride ($\sim 5 \times 10^{-3}$ moles) to a solution containing excess cerium(IV) assures that all the cerium is reduced to the trivalent state. Excess hydroxylamine hydrochloride does not interfere with the polarographic reduction of arsenate (Fig. 22).

This procedure allows the determination of arsenite in the form of arsenate in the absence of arsenate and the determination of total inorganic arsenic (the sum of the arsenite and arsenate concentrations).

Fig. 23 shows calibration curves for the determination of arsenate in the ranges 20 to 160 ppb and 500 to 1500 ppb As(arsenate). These curves were obtained by oxidizing 20 ppb As(arsenite) or 500 ppb As(arsenite) by cerium(IV), reducing excess cerium(IV) by hydroxylamine hydrochloride and recording the polarograms after addition of D-mannitol. Aliquots of

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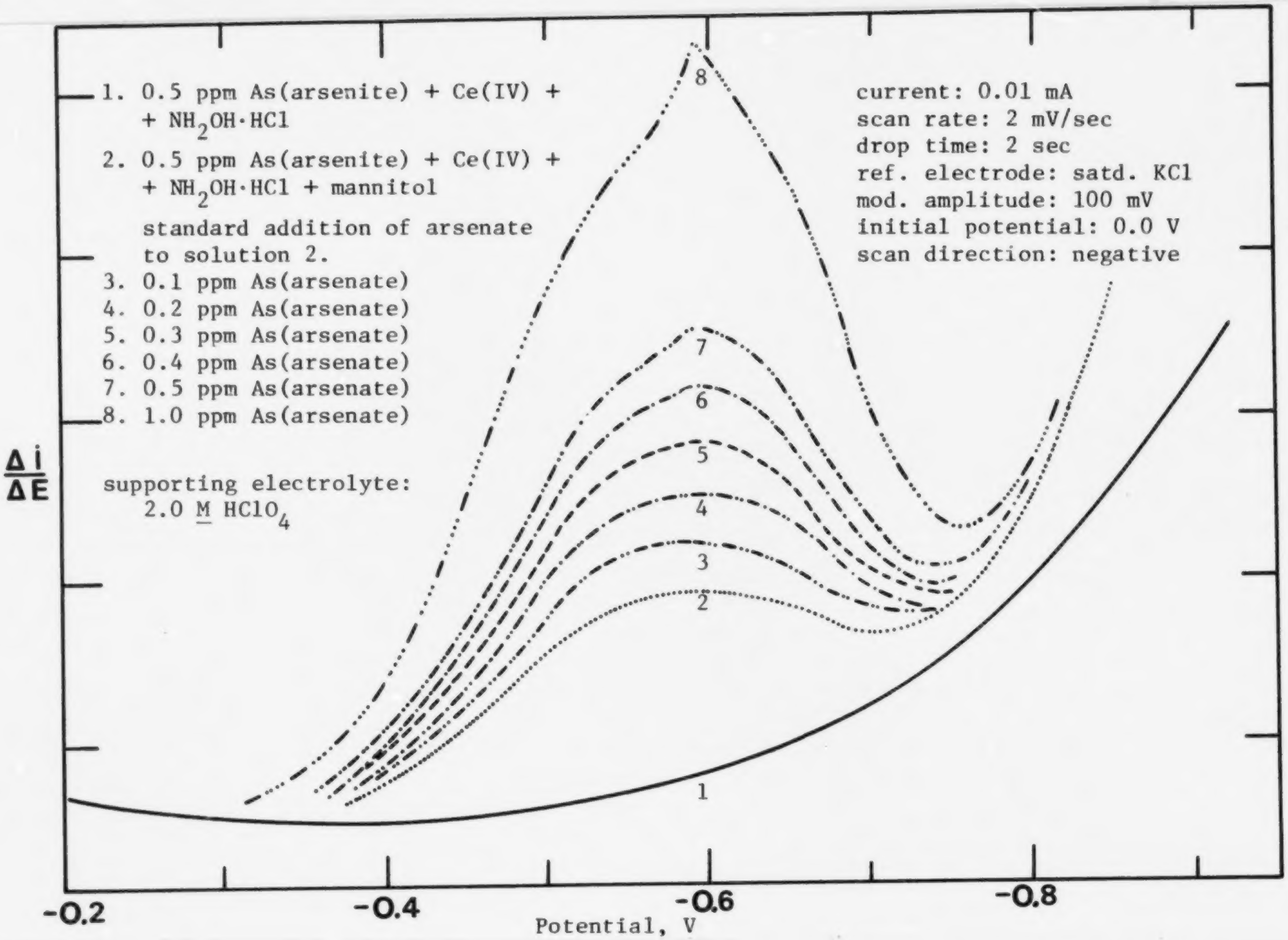


Figure 22. Differential pulse polarograms of 500 ppb As(arsenate) obtained by cerium(IV) oxidation of arsenite in 2.0 $\underline{M}$ perchloric acid, followed by addition of hydroxylamine hydrochloride, addition of $\underline{D}$-mannitol and standard addition of arsenate.

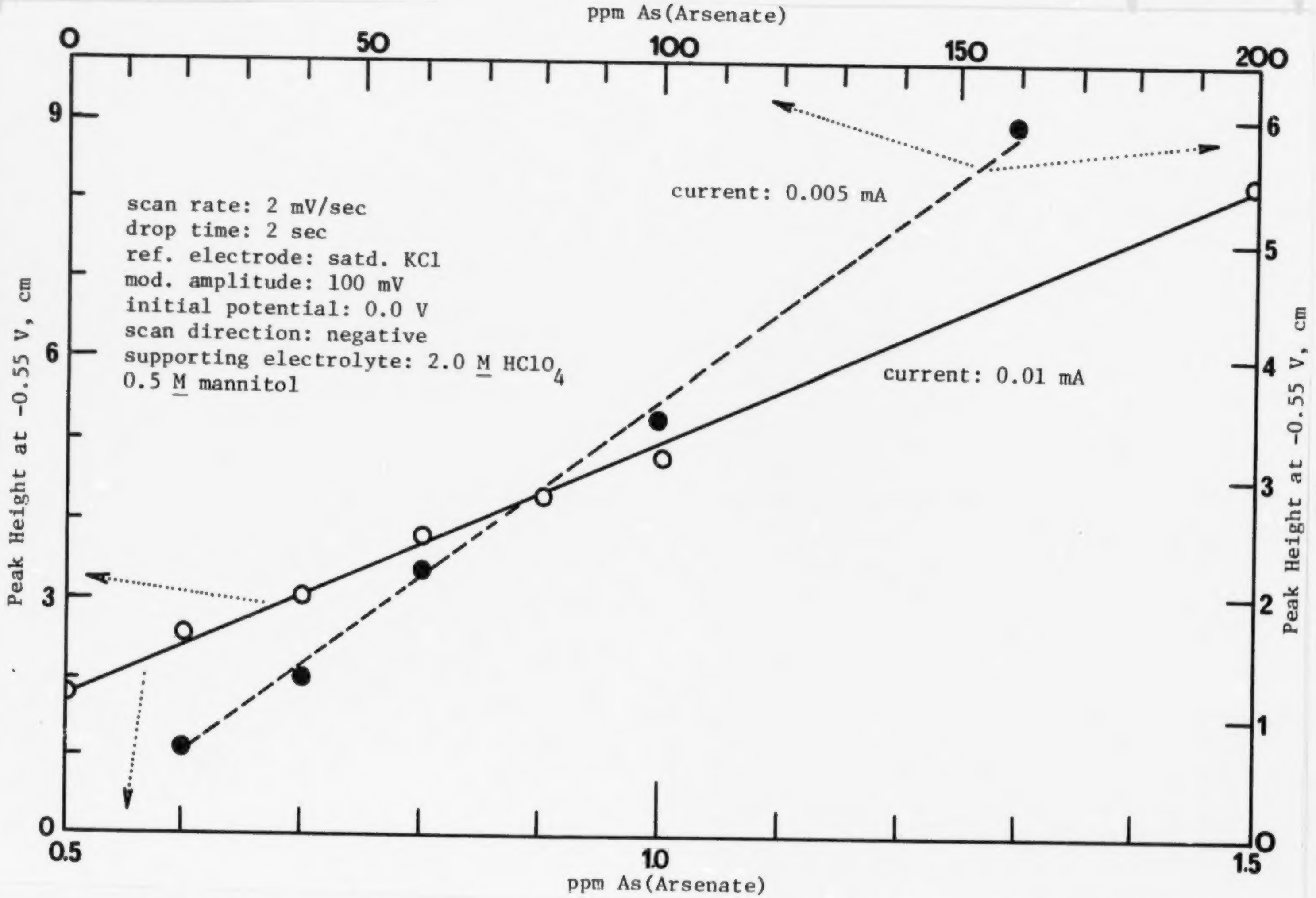


Figure 23. Calibration curves for the determination of arsenate obtained by oxidation of arsenite with cerium(IV).

appropriate standard arsenate solutions were then pipetted into the polarographic cell and the solutions polarographed after each addition. The detection limits for arsenic under these experimental conditions were calculated to be 6 ppb at the 95 percent confidence level and 10.5 ppb at the 99 percent confidence level.

To check the reproducibility of the method, a 200 ppb As(arsenate) solution was polarographed seven times with 2.0 M $HClO_4$ as supporting electrolyte in the presence of mannitol. The peak heights were measured in inches. The mean of the peak heights was 0.543 inches with a standard deviation of 0.012 and a variance of 0.0013.

Based on the experimental results described in the previous sections, the following procedure is recommended for the quantitative determination of arsenate and arsenite in water samples by differential pulse polarography.

1. Pipette 200 mL of the water sample into a 250 mL volumetric flask.
2. Add 42.5 mL of 70 percent perchloric acid and fill to the mark with distilled water.
3. Pipette 50 mL of this solution into the polarographic cell and deaerate for 15 minutes.
4. Determine the arsenite concentration by standard addition employing the maximum at -0.425 V.
5. To another 50 mL aliquot of the sample add 4.5 g D-mannitol, record the polarogram and check for an arsenate reduction peak at -0.55 V.
6. Determine the arsenate concentration by standard addition employing the maximum at -0.55 V if the arsenite and arsenate concentrations permit it.
7. Oxidize arsenite to arsenate with a 200-fold molar excess of cerium(IV) ammonium nitrate by addition of the appropriate number of microliters of a 0.58 M cerium(IV) solution.
8. Reduce the excess cerium(IV) by addition of 0.35 g of hydroxylamine hydrochloride.
9. Record the polarogram and determine arsenate employing the standard addition technique. Measure the peak height at -0.55 V.
10. Subtract the concentration of As(arsenite) obtained in Step 4 from the As(arsenate) concentration determined in Step 9 to find the As(arsenate) concentration originally present in the water sample.

A flow sheet for the recommended analysis procedures is given in Fig. 24. If desired, arsenite can also be determined in another aliquot after addition of *D*-mannitol using the peak at -0.34 V.

Lacking a natural water sample containing both arsenite and arsenate, Texas A&M University tap water was spiked with 100 ppb arsenite and 300 ppb arsenate solutions. In 2.0 *M* perchloric acid 103 ppb As(arsenite) were found in the absence and in the presence of 4.5 g *D*-mannitol. The As(arsenate) concentration was determined as 300 ppb. After oxidation of 50 mL of the spiked tap water sample with cerium(IV) and addition of hydroxylamine hydrochloride 392 ppb total arsenic were detected as arsenate.

Experimental

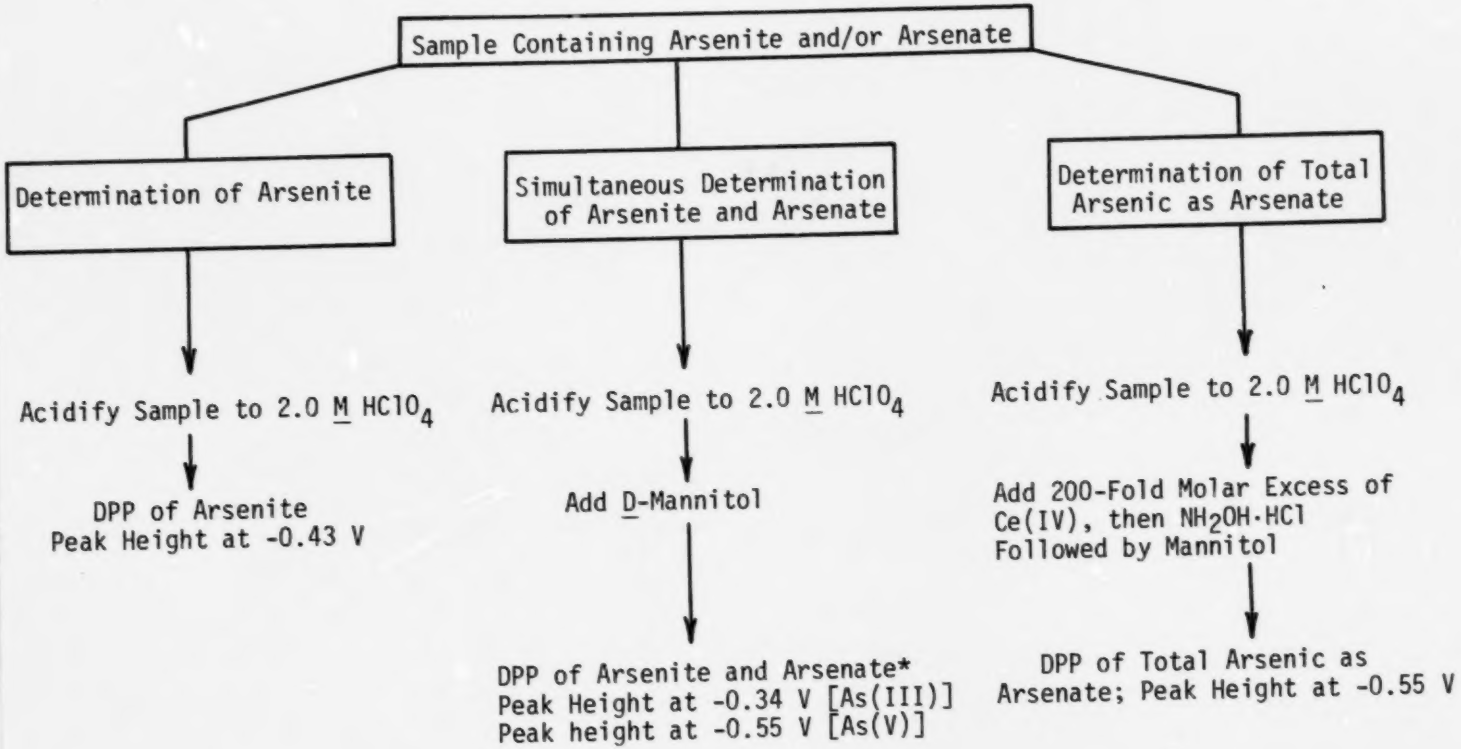
Reagents

The sources of the reagent grade chemicals used in these investigations are listed below.

1,2-Butanediol: Aldrich Chemical Company
2,3-Butanediol: Aldrich Chemical Company
3-Chloro-1,2-propanediol: Aldrich Chemical Company
1,2-Propanediol: Aldrich Chemical Company
Vanadium(III) Chloride: Alfa Division Ventron Corporation
Acetic Acid: Allied Chemical
Hydroxylamine Hydrochloride: American Drug and Chemical Company
Fructose: Eastman Organic Chemicals
Ceric Ammonium Nitrate: Fisher Scientific Company
Ethylene Glycol: Fisher Scientific Company
Hydrochloric Acid: Fisher Scientific Company
Nitric Acid: Fisher Scientific Company
Phosphoric Acid: Fisher Scientific Company
Pyrogallol: Fisher Scientific Company
Disodium Hydrogen Arsenate Heptahydrate: Fisher Scientific Company
Sodium Arsenite ($NaAsO_2$): Fisher Scientific Company
Sucrose: Fisher Scientific Company
Sulfuric Acid: Fisher Scientific Company
Pyrocatechol: J. T. Baker Chemical Company
Zinc: Mallinckrodt, Incorporated
D-Ribose: Pfanstiehl Laboratories, Incorporated
Mercury: The Instrument Grade, triple-distilled mercury was obtained from D. F. Goldsmith Chemical and Metal Corporation.

Instrumentation

All polarograms were recorded employing a PAR (Princeton Applied Research, Incorporated) Model 174A Polarographic Analyzer, with a PAR Model 172A Drop Timer in the differential pulse mode on a Houston Instrument 1000 x-y Recorder.


Sample Containing Arsenite and/or Arsenate
Determination of Arsenite
Simultaneous Determination of Arsenite and Arsenate
Determination of Total Arsenic as Arsenate
Acidify Sample to 2.0 M HClO4
DPP of Arsenite Peak Height at -0.43 V
Acidify Sample to 2.0 M HClO4
Add D-Mannitol
DPP of Arsenite and Arsenate* Peak Height at -0.34 V [As(III)] Peak height at -0.55 V [As(V)]
Acidify Sample to 2.0 M HClO4
Add 200-Fold Molar Excess of Ce(IV), then NH2OH·HCl Followed by Mannitol
DPP of Total Arsenic as Arsenate; Peak Height at -0.55 V

*Determination of arsenate in the presence of arsenite possible only in a restricted range of arsenite and arsenate concentrations and arsenite/arsenate ratios.

Figure 24. Flow chart for the determination of arsenite and arsenate by differential pulse polarography.

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The polarographic cell had a side arm and Luggin probe for the reference electrode. The cell was equipped with a medium porosity gas dispersion tube, a platinum wire counter electrode, and the dropping mercury electrode. A Coleman Model 3-711 Asbestos Wick Saturated Calomel Electrode was used in the side arm as the reference electrode. The calomel electrode must frequently be soaked in distilled water to avoid plugging of the electrode tip by a potassium perchlorate precipitate.

Degassing of Solutions

Nitrogen was passed through two scrubbing towers each containing an aqueous solution of vanadium(III) chloride over amalgamated zinc. Before entering the cell the nitrogen was passed through a presaturation tower containing the supporting electrolyte. The cell contents were degassed for 15 minutes by passing the scrubbed nitrogen through the solution. During the polarographic determination nitrogen was passed over the surface of the solution.

General Procedure for Sample Preparation

The stock solutions of arsenite and arsenate were prepared fresh for each analysis using $NaAsO_2$ and $Na_2HAsO_4 \cdot 7H_2O$, respectively. The concentrations of these stock solutions were chosen in such a manner that the addition of 20 microliters to the contents of the polarographic cell would produce the desired concentration. For example, 20 microliters of a 2500 ppm As(arsenite) solution pipetted into a 50 mL solution in the polarographic cell produced a 1 ppm As(arsenite) solution. The pipette used was a fixed volume Eppendorf micropipette.

Standard Addition

The method of Standard Additions was used in the analyses for arsenite and arsenate. The additions were made by pipetting microliter aliquots of the arsenite and arsenate solution into the side arm of the polarographic cell. All of the solution in the side arm was then forced into the main chamber of the cell by applying air pressure by means of a pipette bulb.

Preparation of Natural Water Samples for Analyses

A 200-mL aliquot of the natural water sample was pipetted into a 250-mL volumetric flask. Then 42.5 mL of 70% $HClO_4$ were added. Distilled, deionized water was used to fill the flask to the mark.

Supporting Electrolytes

The following acids were used as supporting electrolytes: sulfuric, hydrochloric, acetic, phosphoric, and perchloric. The concentration of the supporting electrolytes was made 2.0 $\underline{M}$ with the single exception of perchloric which was prepared in the range from 0.7 $\underline{M}$ to 7 $\underline{M}$. The supporting electrolytes were prepared by adding the appropriate amount of acid to deionized distilled water. For example, 8.5 mL of 70% (12 $\underline{M}$) perchloric acid were pipetted into a 50 mL volumetric flask containing distilled,

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deionized water, the contents mixed, and the flask filled to the mark with distilled, deionized water.

Polyhydroxy Compounds

The following polyhydroxy compounds were used as reagents: 3-chloro-1,2-propanediol, pyrocatechol, pyrogallol, 1,2-ethanediol, 1,2-propanediol, 1,2-butanediol, D-ribose, sucrose, fructose, 2,3-butanediol, and D-mannitol. The concentrations of the polyhydroxy compounds were 0.5 M. They were weighed only with one-tenth gram accuracy. For example, 4.5 g of D-mannitol were added to 50 mL of solution in the polarographic cell. The mixture was stirred until all the mannitol had dissolved. The resulting solution was approximately 0.5 M with respect to mannitol.

MASS SPECTROMETRY OF ORGANYLARSONIC ACIDS, DIORGANYLARSINIC ACIDS, ORGANYLIODOARSINES, ARSENOCHOLINE AND ARSENOBETAINE

Mass spectrometry has the advantage of high sensitivity, of requiring only small samples and of providing both elemental composition and structural information. High resolution mass spectrometry will also distinguish between substances containing only lighter elements and those having one or more heavy elements in their molecules.

Arsinic and arsonic acids could perhaps be determined by mass spectral analysis in the residues obtained by evaporation of water samples. The mass spectral behavior of five organylarsonic acids and fourteen diorganylarsinic acids was studied using a CEC 21-110B mass spectrometer.

At probe temperatures between 110° and 250° required to obtain satisfactory mass spectra, the arsinic and arsonic acids form anhydrides and decompose. The products of these thermal reactions are then ionized and fragmented yielding complicated mass spectra with many peaks at m/e values higher than those expected for the molecular ions of the arsinic and arsonic acids (Figs. 25, 26).

There are several reports in the literature concerning the dehydration of arsonic acids. Differential thermal and vacuum thermogravimetric experiments indicated that arsonic acids, $RAsO_3H_2$, are first converted to diarsonic acids, $HO_2(R)As{-}O{-}As(R)O_2H$, and then via polyarsonic acids to the polymeric arsonic acid anhydrides, $(RAsO_2)_n$, which decompose on further heating to arsenic trioxide (67). Arsinic acids, R_2AsOOH, can only form the anhydrides, $[R_2As(O)]_2O$.

The mass spectra of the arsonic acids do not contain molecular ions for the acids, the diacids or the anhydrides. The highest mass peak in the spectrum of methylarsonic acid corresponds to $CH_3As_4O_6$. In the spectra of most of the acids As_4O_6 is the highest mass peak. Characteristic fragments are formed by loss of arsenic and oxygen from As_4O_6 and by loss of methyl, oxygen and hydrogen from CH_3AsO_3H. In all the spectra peaks of considerable intensity are observed for As_n (n = 1-4). The arsenic is

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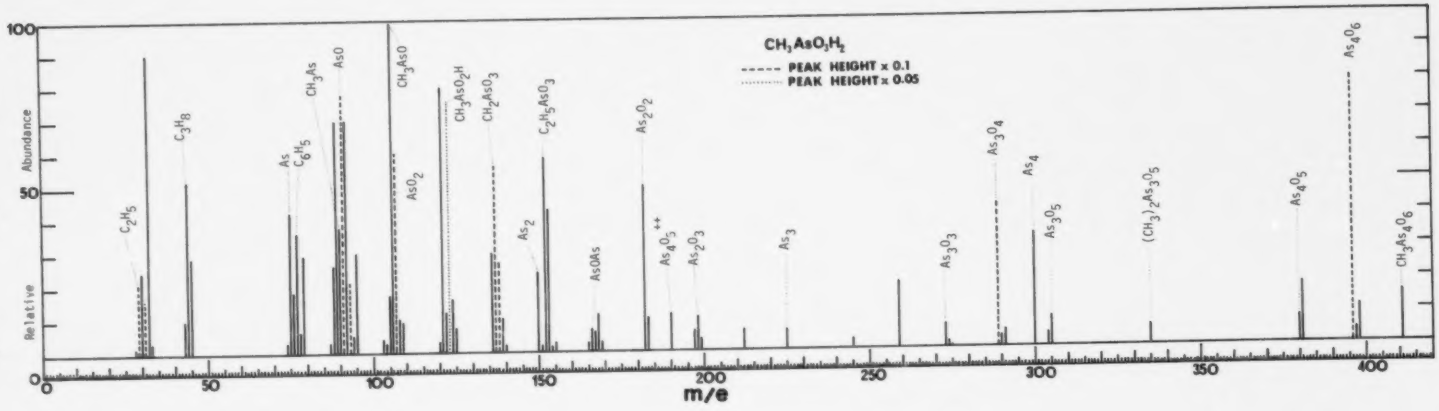

Figure 25. The mass spectrum of methylarsonic acid.

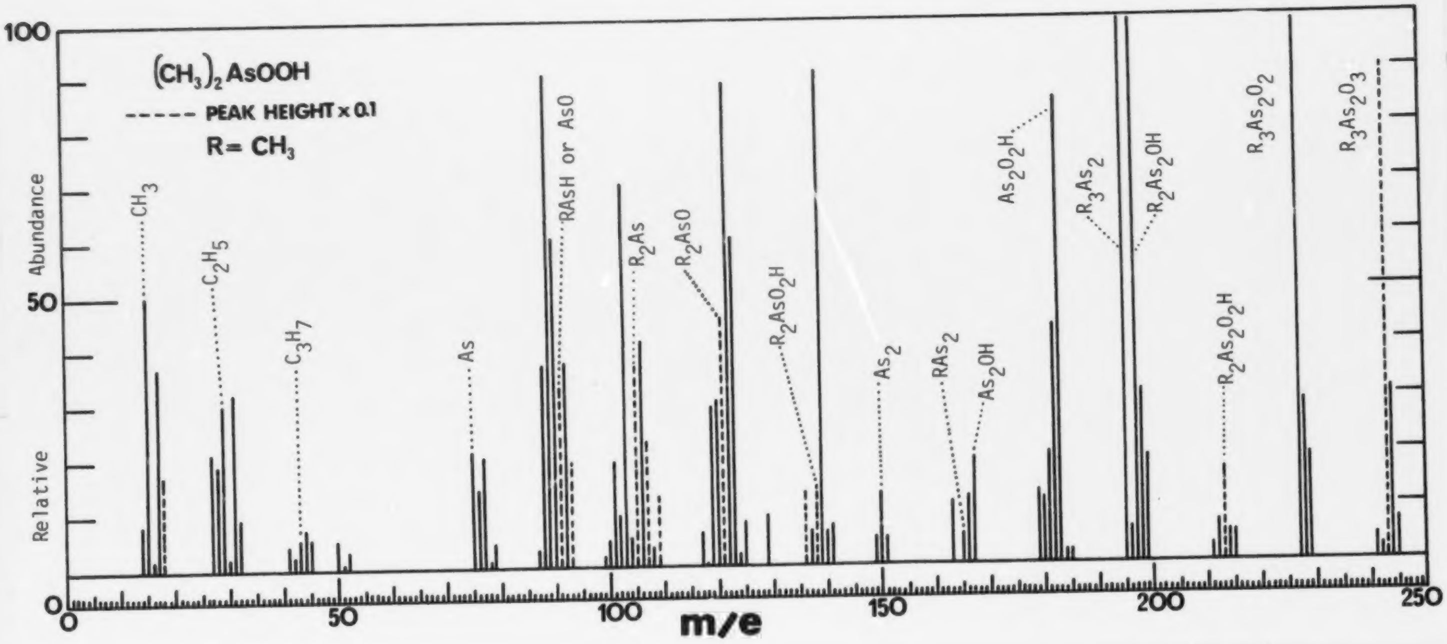

Figure 26. The mass spectrum of dimethylarsinic acid.

probably formed by decomposition of arsenic-containing materials on the hot filaments in the ion source. The most important ions in the mass spectra of arsonic acids are summarized in Table 4.

Diorganylarsinic acids appear to produce molecular ions (Fig. 26, Table 5). The signals for these ions decrease with increasing molecular mass of the arsinic acid. The ions with m/e values equal to or lower than those of the molecular ions and the ions containing arsenic and oxygen only are listed in Table 5. Molecular ions formed from arsinic acid anhydrides have not been observed in any of the mass spectra. However, intense peaks corresponding to $R_3As_2O_3$ are present in the spectra. The signals with m/e values higher than those of the molecular ions of R_2AsOOH are generated by fragments arising through loss of alkyl groups, oxygen, hydroxyl groups and hydrogen from $R_3As_2O_3$.

Mass spectrometry is of little value for the identification of arsonic acids, but can be used to establish the presence of diorganylarsinic acids in the residues obtained from water samples. Exact mass measurements by high resolution mass spectrometry might be necessary to distinguish arsenic-containing from arsenic-free fragments.

Organyliodoarsines, R_nAsI_{3-n} (n = 1,2) are much more volatile than arsinic or arsonic acids, are not capable of forming anhydrides and are easily prepared by treating arsinic and arsonic acids with hydriodic acid (68). These reactions should be useful for the conversion of organylarsenic acids in environmental samples to organyliodoarsines, which can then be determined by mass spectrometry. Arsenite and arsenate are both transformed to arsenic triiodide under these conditions.

Purified samples of organyliodoarsines were employed to obtain their mass spectra at probe and source temperatures given in Tables 6 and 7. All the iodoarsines gave intense molecular ion peaks. Fragmentation proceeds by loss of alkyl groups, iodine and hydrogen abstraction. Peaks of lower intensity corresponding to AsI_3, R_2AsI and As_n (n = 1-4) were observed in many of the spectra. Thermally induced disproportionation of RasI into R_2AsI and AsI_3 is a probable mechanism for the formation of these compounds. Interesting series of fragments represented by the formulas $C_nH_{2n+1}AsI$, $C_nH_{2n+2}As$ and $C_nH_{2n}As$ are part of the mass spectra of organyliodoarsines. It appears that methylene groups are lost generating signals fourteen mass units apart from n = 0 to n equal to the number of carbon atoms in the organyliodoarsines. Additional experiments are in progress to verify this unusual cleavage of methylene. As examples for the mass spectra of organyliodoarsines the spectra of butyldiiodoarsine and dioctyliodoarsines are presented in Fig. 27 and Fig. 28, respectively. Organyliodoarsines are well suited for the mass spectrometric identification of organic arsenic compounds which can be converted to their iodo derivatives.

Arsenocholine chloride $[(CH_3)_3AsCH_2CH_2OH]^+Cl^-$, (Fig. 29) and arsenobetaine chloride, $[(CH_3)_3AsCH_2COOH]^+Cl$, (Fig. 30) produce rich mass spectra, which, however, do not contain molecular ion peaks. The highest mass peaks correspond to $(CH_3)_3AsCH_2CH_2O$ and $(CH_3)_2AsCH_2COOH$, which are

TABLE 4. RELATIVE ABUNDANCE OF IONS IN THE ELECTRON IMPACT MASS SPECTRA OF ORGANYLARSONIC ACIDS

	R in $RAsO_3H$				
Ion	CH_3	C_2H_5	C_3H_7	C_9H_{19}	C_6H_5
R	0.00	11.32	100.00	0.38	31.43
RAs	3.60	0.40	0.57	7.30	21.43
$RAsO_2$	1.15	3.40	0.64	1.03	0.00
$RAsO_2H$	100.00	100.00	29.90	0.23	0.00
$RAsO_3$	24.04	0.00	0.50	0.00	0.00
$RAsO_3H$	0.96	1.21	0.64	0.00	0.00
$RAsO_3H_2$	0.00	0.16	0.00	0.00	0.00
$RAsO_3H_3$	8.30	8.29	2.36	0.00	0.00
R_2As	1.63	0.00	0.00	2.40	19.29
R_2AsO	7.20	0.58	0.00	0.34	3.29
R_2AsO_2	53.90	18.29	5.36	0.00	11.43
R_2AsO_2H	24.00	7.63	1.57	0.00	3.00
$R_2AsO_2H_2$	4.00	3.95	1.64	0.00	1.71
As	4.40	1.84	2.86	0.34	4.71
AsO	75.00	53.95	54.29	0.91	80.71
AsO_2	56.70	0.53	3.29	0.11	0.00
AsO_3	-	0.89	4.79	1.14	0.43
As_2O_2	4.70	3.80	3.43	0.11	9.29
As_2O_3	0.96	0.76	0.57	5.14	0.00
As_3O_4	40.38	19.00	9.29	0.80	62.86
As_4O_6	75.96	21.60	8.57	2.40	92.86
Probe Temp °C	200	165	180	210	255
Source Temp °C	210	235	210	230	210

TABLE 5. RELATIVE ABUNDANCES OF IONS IN THE ELECTRON IMPACT MASS SPECTRA OF DIORGANYLARSINIC ACIDS

	R in R_2AsOOH								
Ion	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	$c\text{-}C_6H_{11}$	C_8H_{17}	C_6H_5	$C_6H_5CH_2CH_2$
R	5.56	0.00	3.48	5.00	4.00	100.00	1.19	18.80	100.00
RAs	7.22	0.96	1.96	3.11	5.00	0.00	17.84	25.56	24.27
RAsO	4.56	0.93	0.43	2.30	3.17	0.00	4.83	2.33	0.78
$RAsO_2$	8.33	0.00	0.30	0.22	0.67	0.00	12.27	0.33	0.00
$RAsO_2H$	6.67	0.37	0.30	0.32	0.50	0.00	4.46	0.00	0.00
$RAsO_3H$	0.56	0.00	0.07	0.00	0.00	0.00	0.52	0.00	0.00
$RAsO_3H_3$	0.77	0.00	0.20	11.89	0.00	0.00	1.86	0.00	0.00
R_2As	40.56	1.00	0.50	0.22	0.33	0.00	1.64	46.94	0.00
R_2AsO	51.11	2.11	0.39	1.62	1.98	9.77	1.78	23.89	0.00
R_2AsO_2	0.67	1.15	1.17	0.00	0.00	4.65	0.74	14.44	0.00
R_2AsO_2H	15.56	7.41	0.20	0.11	0.00	4.65	6.15	3.33	0.00
$R_2AsO_2H_2$	8.89	0.44	5.87	0.49	0.00	23.26	4.01	5.28	0.00
As	2.33	0.33	0.02	0.03	0.25	1.16	0.37	1.28	0.58
AsO	36.67	10.37	11.02	2.30	3.00	1.63	5.58	10.56	9.90
AsO_2	26.67	0.11	1.24	1.11	1.42	1.86	3.94	0.00	0.78
AsO_3	6.67	0.00	0.04	0.00	0.00	0.00	0.52	0.00	0.00
As_2O_2	4.56	20.37	2.61	2.30	2.08	1.16	0.89	3.89	3.50
As_2O_3	3.33	0.33	0.28	0.24	0.25	0.00	0.97	0.00	0.00
As_3O_4	0.00	2.22	0.91	1.62	1.42	0.93	2.68	1.83	12.62
As_4O_6	0.00	1.89	1.30	0.92	10.00	0.70	8.18	6.33	18.45
Probe Temp °C	184	110	120	130	165	150	202	220	220
Source Temp °C	210	200	200	220	210	210	210	220	220

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TABLE 6. RELATIVE ABUNDANCES OF IONS IN THE ELECTRON IMPACT MASS SPECTRA OF ORGANYLDIIODOARSINES

	R in $RAsI_2$								
Ion	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_7H_{15}	$C_{14}H_{29}$	C_6H_5	$C_6H_5CH_2CH_2$
R_2AsI	0.19	0.15	0.14	0.24	0.30	21.05	3.23	0.03	0.26
$RAsI_2$	100.00	100.00	100.00	57.58	65.57	7.02	35.48	31.03	2.30
RAsI	51.58	48.75	23.88	13.33	21.52	7.02	100.00	100.00	57.45
AsI_2	11.84	24.88	32.24	19.39	24.78	6.14	85.48	2.53	2.77
AsI	1.42	31.86	41.63	20.61	20.87	7.50	30.65	2.64	3.19
R_2As	0.08	0.06	0.05	---	0.04	2.10	0.09	0.14	0.18
RAs	10.53	3.25	2.45	4.24	3.48	37.74	6.77	30.34	31.06
AsI_3	0.10	0.31	0.86	0.18	0.83	---	---	2.76	0.91
I	13.95	16.86	16.73	9.09	7.83	4.34	48.39	4.60	5.74
As	3.42	7.81	4.29	3.21	2.83	0.87	5.00	0.92	0.98
R	0.47	9.69	61.22	100.00	63.04	1.66	14.35	0.60	100.00
I_2	2.92	1.31	1.69	0.72	0.69	0.46	0.32	1.84	1.96
Probe Temp °C	40	45	30	40	35	60	85	50	55
Source Temp °C	220	190	190	210	210	190	250	200	200

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TABLE 7. RELATIVE ABUNDANCES OF IONS IN THE ELECTRON IMPACT MASS SPECTRA OF DIORGANYLIODOARSINES

	R in R_2AsI				
Ion	CH_3	c-C_6H_{11}	C_8H_{17}	$C_{10}H_{22}$	$C_6H_5CH_2CH_2$
R_2AsI	100.00	25.24	35.96	44.96	6.82
$RAsI_2$	0.41	0.10	1.05	3.88	---
RAsI	63.84	12.38	3.93	8.37	1.36
AsI_2	0.32	0.14	2.13	4.42	0.07
AsI	16.07	1.31	7.30	6.36	1.23
R_2As	1.09	1.21	3.47	5.81	97.73
RAs	61.61	3.33	100.00	100.00	97.73
As	8.04	0.21	1.01	0.62	0.93
R	3.57	100.00	4.83	4.19	100.00
I	15.18	2.00	5.39	4.57	2.05
I_2	0.15	0.29	0.20	0.23	0.07

formed by thermal cleavage of HCl and CH_3Cl from the arsonium salts. Fragmentation proceeds by loss of methyl groups, cleavage of hydroxyl groups and loss of ethylene. Fragments containing arsenic-oxygen or arsenic-chlorine bonds were either formed by thermal decomposition of the arsonium salts or through intramolecular rearrangements. In spite of the absence of molecular ions mass spectrometry can provide an indication of the presence of arsenocholine or arsenobetaine in a sample.

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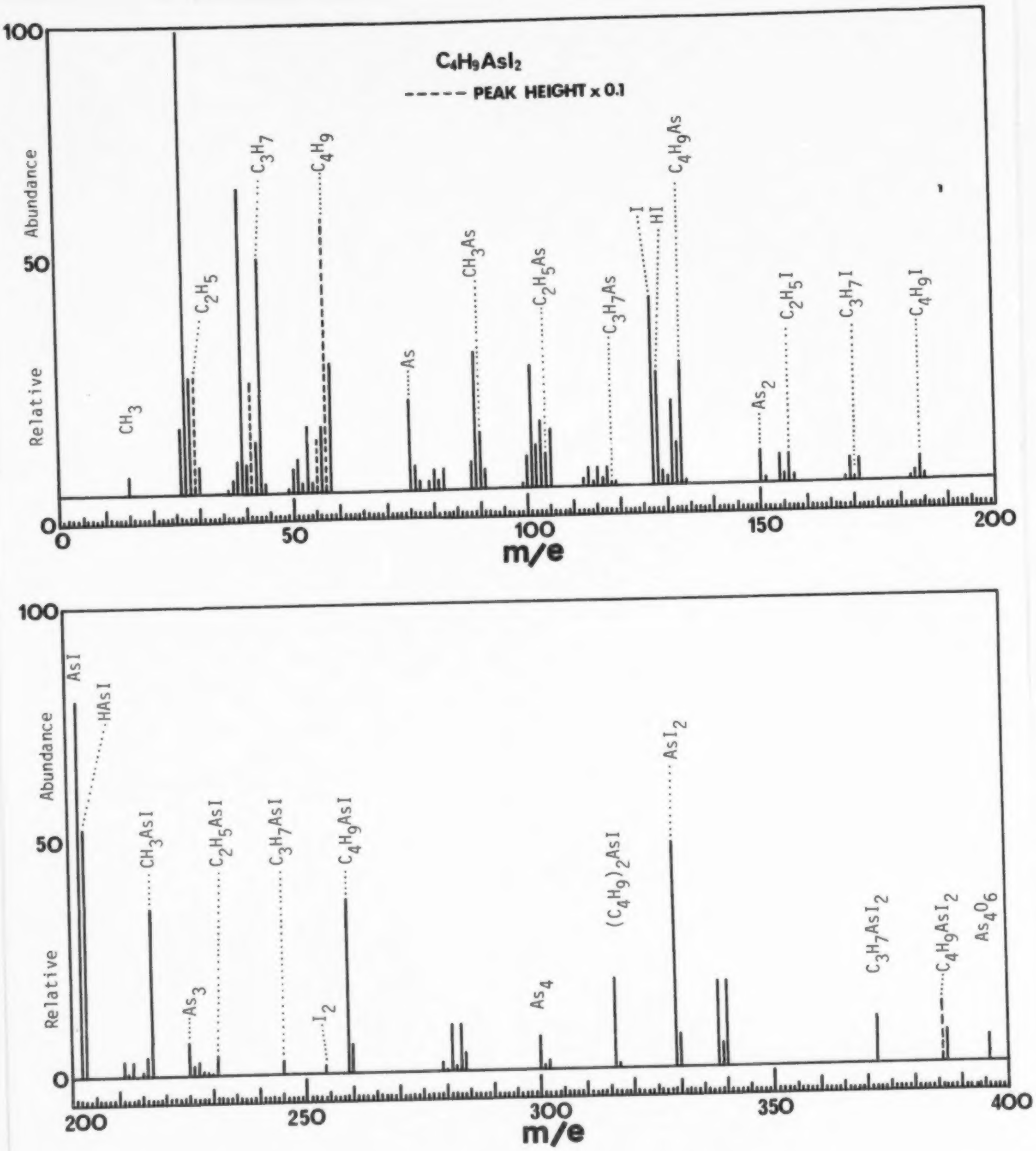

Figure 27. The mass spectrum of butyldiiodoarsine.

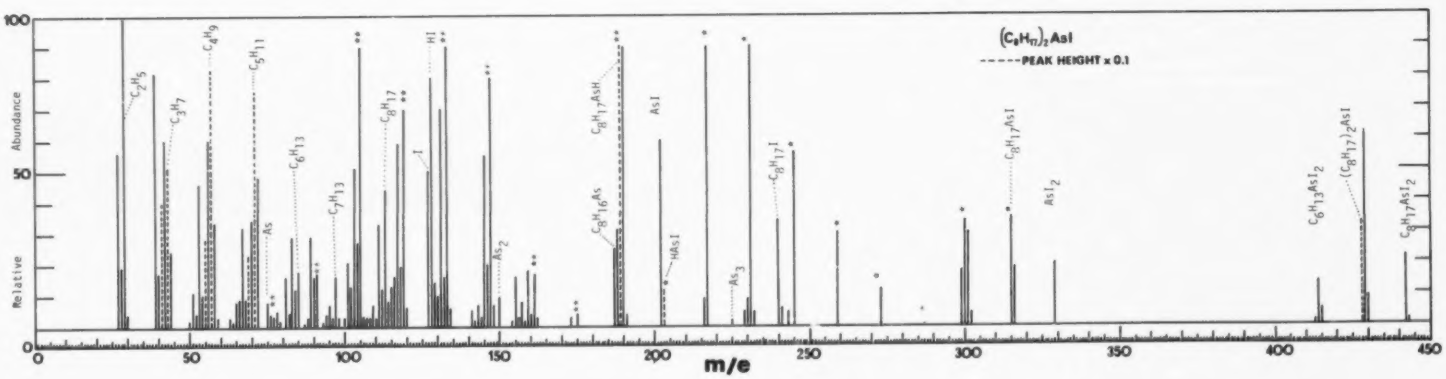

Figure 28. The mass spectrum of dioctyliodoarsine. The peaks marked by * and ** belong to the series $C_nH_{2n+1}AsI$ and $C_nH_{2n+2}As$, respectively.

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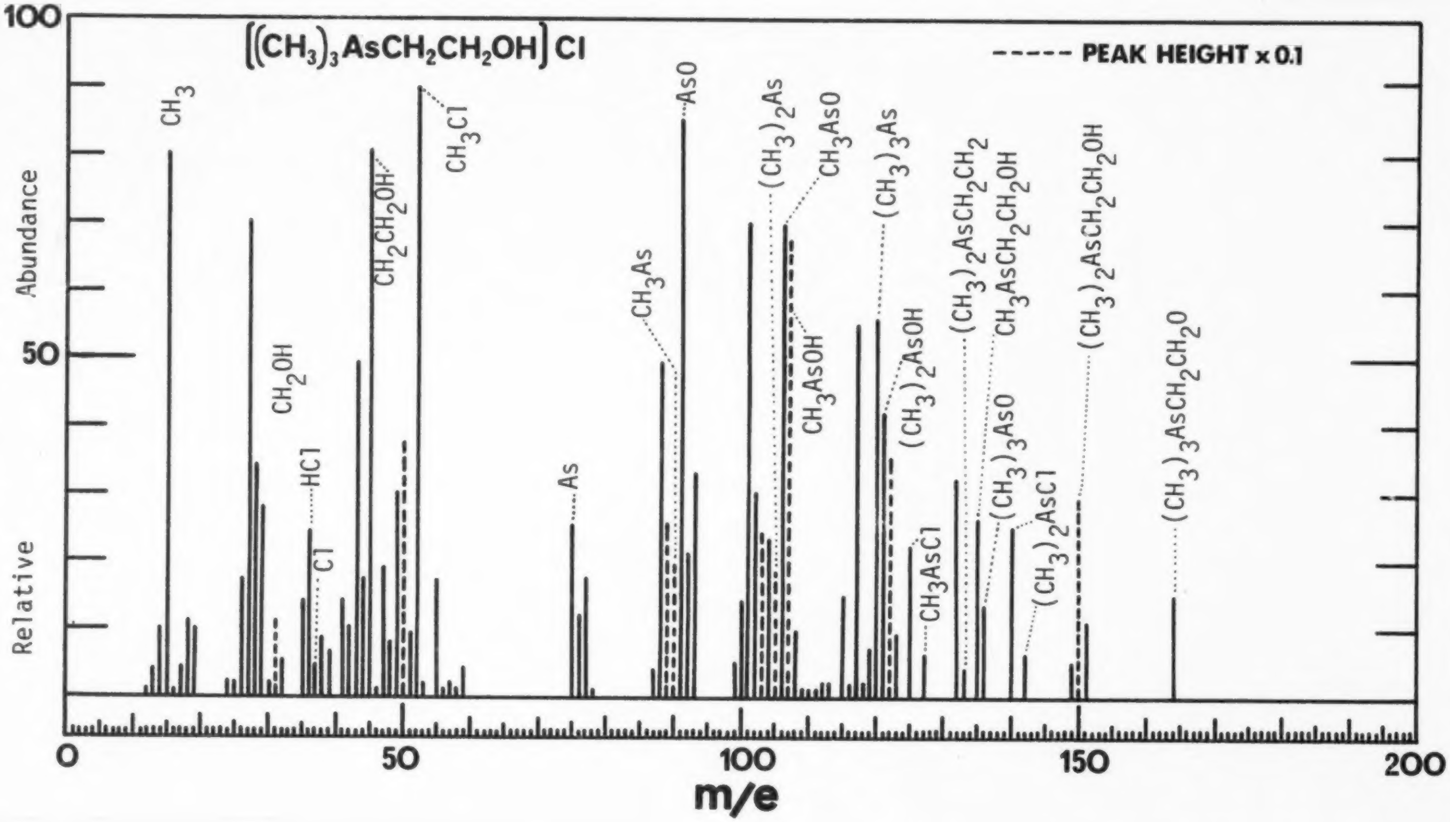

Figure 29. The mass spectrum of arsenocholine chloride.

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TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
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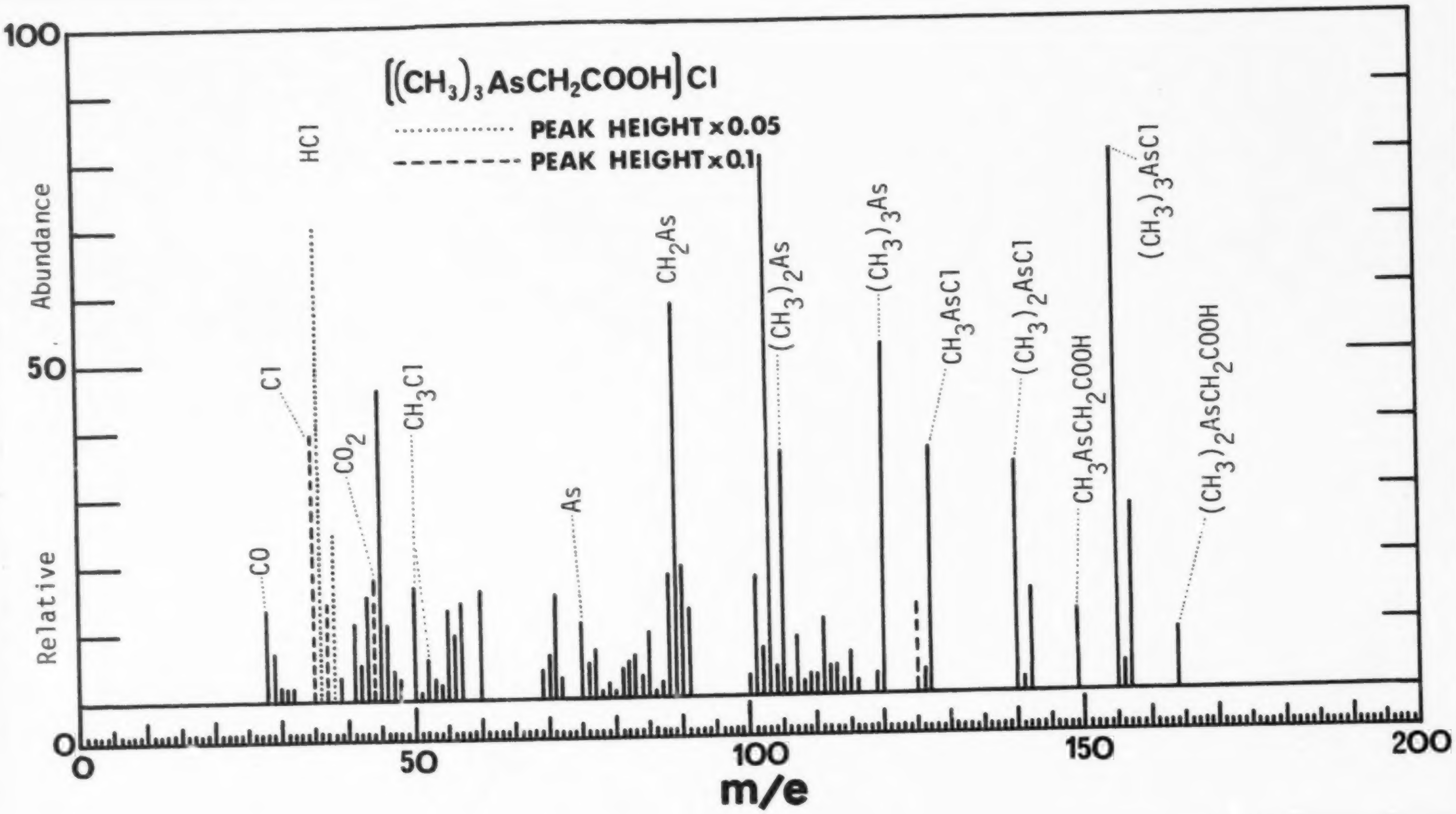

Figure 30. The mass spectrum of arsenobetaine chloride.

SECTION 7

ANALYSIS OF WATER SAMPLES

Samples of arsenic-containing drinking water supplies selected by the project officer were collected and shipped to College Station as quickly as possible. Total arsenic concentrations and the concentrations of arsenite and arsenate were determined by several methods. Each water sample was checked for the presence of methylated arsenic compounds and other organic arsenic derivatives. Water samples from Hinckley, Utah; Delta, Utah; Barefoot Site, Alaska; Mauer Site, Alaska; Antofagasta, Chile; Yenshei, Taiwan; Hartlin Site, Nova Scotia; and Sullivan Site, Nova Scotia were investigated.

The following methods were employed for the determination of total arsenic and arsenic compounds.

- Graphite furnace atomic absorption spectrometry: A Hitachi Zeeman graphite furnace atomic absorption spectrometer with graphite cups was employed for total arsenic determinations. Forty microliters were injected in most cases. Since GFAA is subject to many interferences arsenic was extracted from water samples. The extract was then injected into the graphite cup.

  A suitable aliquot of the water sample is made 0.1 $\underline{M}$ with respect to hydrochloric acid. Arsenate is reduced to arsenite by addition of 5 mL of a solution containing $NaHSO_3$, $Na_2S_2O_3$ and HCl. The solution is extracted with 5 mL of 0.01 $\underline{M}$ $(C_4H_9O)_2PS_2H$ in hexane. The extraction is repeated four times with 2 mL each of the reagent solution. The combined organic layer is washed first with 4.5 $\underline{M}$ HCl and then with water. The organic layer is extracted twice with 2 mL of bromine water. The aqueous phases are transferred to a volumetric flask, adjusted to 0.1 $\underline{M}$ acid with nitric acid, brought to 200 ppm with respect to nickel by addition of nickel nitrate and treated with 50 µL of 30% hydrogen peroxide. After filling to the mark with distilled water, 20 µL are injected into the graphite cup for analysis. The standards and blanks are prepared in the same manner.

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- Differential pulse polarography: Arsenite, arsenate and total arsenic were determined by DDP as described in Section 6.

- High pressure liquid chromatography/GFAA as element-specific detector: Concentrated water samples were analyzed by the HPLC-GFAA method to check for the presence of organic arsenic compounds as described in Section 6.

- Hydride generation technique: Arsenite, arsenate and methylated arsenic compounds are reduced by sodium borohydride at pH 1. At pH 6.5 only arsenite is reduced. The reductions are carried out in a chamber capable of holding 10 mL of sample. After the addition of the appropriate buffer solution a measured volume of sodium borohydride solution is pumped into the chamber. A stream of helium transports the arsines through a U-trap cooled in an isopropanol/dry ice bath to remove water. The arsines are collected in a U-trap cooled by liquid nitrogen. One leg of this trap is packed with silanized glass beads. After the arsines had been transferred to the U-trap, they are rapidly volatilized by electrically heating the trap. A stream of helium transports the arsines through a short tube packed with sodium hydroxide pellets to remove carbon dioxide into the helium discharge created by passing direct current between two helium-arc welding electrodes. The radiation from the discharge passes through the quartz window of the emission chamber and is focused on the entrance slit of the monochromator set at the 228.8 nm arsenic emission line. A gas chromatograph can be attached to the exit of the arsine trap to improve separation of AsH_3 from methylarsines. The hydride generation technique has detection limits of 0.2 ppb for arsine, 0.3 ppb for methylarsine and 1 ppb for dimethylarsine.

- Inductively coupled argon plasma emission spectrometry: ICP is the most convenient method to determine the trace element concentrations including arsenic concentrations in water samples. An ICP spectrometer became available at Texas A&M University during the last months of the project. The recently collected samples were analyzed for 48 elements by the simultaneous ICP Model 34000 manufactured by ARL. Prior to this time ICP analyses were carried out with ARL instruments at Radian Corporation in Austin, Texas or at the ARL plant in Sunland, California.

As an illustrative example for the sampling procedures the sampling instructions for the Taiwan well waters are cited. Sampling instructions

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were pasted to each bottle. The correct amounts of preservations in vials were also attached to the bottles (with exceptions of nitric acid).

Rinsing of all bottles before filling:

1. Fill bottle half full with well water, close bottle with cap and shake thoroughly. Empty bottle.
2. Repeat procedure of item 1 three times.
3. Fill the bottle as described below.
4. Tightly close the cap, wipe it dry and tape securely with the tape provided.

Glass container for unpreserved sample (Well I):

Fill the rinsed bottle with water to the brim, close the cap tightly and secure by taping the cap with the tape provided. Provide the information requested on the label.

Glass container for sample preserved with $HgCl_2$ (Well I):

Fill the rinsed bottle with water almost to the brim. Add all of the mercuric chloride in the vial to the sample. Fill to the brim, close the cap tightly and secure by taping the cap. Shake the closed bottle to dissolve the mercury salt. Provide the information requested on the label.

Plastic containers for unpreserved sample (2 containers for Well I; 2 containers for Well II):

Fill the rinsed bottles with water to the brim. Make sure no air space remains in the bottle. Close the cap tightly and tape. Provide the information requested on the label.

Plastic containers for sample preserved with 0.1 weight % ascorbic acid (2 containers for Well I; 2 containers for Well II):

Fill the rinsed bottles with water almost to the brim. Add all the ascorbic acid from the vial to the sample. Fill to the brim, close the cap tightly and secure by taping. Turn the bottles several times to aid the dissolution of ascorbic acid. Provide the information requested on the label.

Plastic containers for samples preserved with nitric acid (2 containers for Well I; 2 containers for Well II):

Fill the container almost to the brim. Add 7 mL of ultrapure nitric acid. Fill to the brim, close the cap and secure by taping. Shake the bottle several times to make the sample homogeneous. Provide the information requested on the label.

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Nitric Acid Control Solution:

Measure 7.0 mL concentrated nitric acid from the nitric acid bottle used for the preservation of the water sample into a clean 1.0 L volumetric flask. Fill to the mark with distilled water and shake to homogenize the solution. Fill the two 125 mL-plastic bottles with this solution, tighten the cap securely and tape the cap.

The results of the analyses of the water samples are presented in the following sections.

The samples were shipped in sturdy cardboard or wooden boxes. None of the samples were shipped frozen.

WATER SAMPLES FROM HINCKLEY AND DELTA, UTAH

Water samples from Delta and Hinckley, Utah were collected by Mr. E. Western, Utah State Department of Health, Division of Environmental Health on July 16, 1979. Pyrex glass bottles and Cubitainers were employed as containers. Some of the samples were preserved by addition of 0.1 weight percent of ascorbic acid. The samples were shipped to College Station as air freight and received July 17, 1979. The analyses were performed during the period July 17 to 25, 1979.

The following analytical methods were employed: graphite furnace atomic absorption spectrometry (GFAA) using a Hitachi-Zeeman GFAA Model 170-70; differential pulse polarography (Princeton Applied Research Model 174A); hydride generation with atomic emission spectrometric detector (Braman method); inductively coupled argon plasma emission spectrometry (ARL 34,000).

Hinckley Water Samples

The results of the total arsenic analyses of the Hinckley water samples are listed in Table 8. The average arsenic concentration in the Hinckley water samples obtained from 36 determinations (twelve each by graphite furnace atomic absorption spectrometry, differential pulse polarography and hydride generation) is 185±8 ppb.

The analyses of the water samples by ICP were carried out several weeks after the collection, because the instrument had not been available earlier. The samples stored in plastic containers gave ICP arsenic concentrations of 170 ppb (without ascorbic acid) and 160 ppb (with ascorbic acid), which are in fair agreement withthe average value from the other methods. The ICP values in glass containers (140 ppb and 120 ppb) were considerably lower than the values obtained from samples stored in plastic containers. Glass containers in contrast to polyethylene containers are known to adsorb arsenic on prolonged storage (see section 5). Samples stored in glass containers are, therefore, expected to have less arsenic in solution than fresh samples or samples stored in plastic Cubitainers.

TABLE 8. TOTAL ARSENIC CONCENTRATIONS IN ppb IN THE HINCKLEY AND DELTA WATER SAMPLES

Location	Ascorbic Acid	Plastic Container	Pyrex Container	GFAA†	DPP†	HG†	ICP†
Hinckley	-	+	-	183±5*	200±10*	185±2*	170
	+	+	-	175±5*	189±10*	183±1*	160
	-	-	+	166±5*	189±10*	178±7*	140
	+	-	+	172±5*	196±22*	200±1*	120
Delta	-	+	-	22±2*	36±4*	18±1*	<80
	+	+	-	24±3*	--	18±1*	<80
	-	-	+	19±1*	24±4*	18±1*	<80
	+	-	+	22±2*	18±1*	17±1*	<80

*Average deviation ± average deviation of three determinations per sample.

† GFAA: Hitachi zeeman graphite furnace atomic absorption spectrometry.
DPP: Differential pulse polarography.
HG: Hydride generation (Braman method).
ICP: Inductively coupled argon plasma emission spectrometry (detection limit for As: 60 ppb).

The determinations of arsenic compounds were performed employing the hydride generation (HG) technique and differential pulse polarogrphy (DPP). The results are summarized in Table 9. Only arsenite and arsenate were found. The average concentration of arsenate was 172±7 ppb (by HG) and 187±8 ppb (DPP). The arsenite concentrations averaged 11 ppb. The arsenite concentrations (by HG) in the samples preserved with ascorbic acid were approximately four times higher than in the unpreserved samples. Ascorbic acid might have reduced some arsenate (see section 5) at these low arsenic concentrations. The sum of the concentrations of arsenite and arsenate agree well with the values of the total arsenic concentrations which were determined separately.

Organic arsenic compounds, if present at all, cannot occur at concentrations larger than a few part per billion. The search for methylated arsenic compounds by the hydride generation method, which has detection limits for such compounds of approximately 1 ppb did not produce any indication of their presence.

The results of the ICP analyses of the Hinckley water samples are summarized in Table 10. With the exception of the concentrations of As, B, Ba and Si the listed values are averages obtained from the concentrations of the following four samples: Pyrex container with 0.1% ascorbic acid; Pyrex container without ascorbic acid; plastic Cubitainer with 0.1% ascorbic acid; plastic Cubitainer without ascorbic acid. The values for As, B, Ba and Si are averages of the samples stored in Cubitainers. The elements present in concentrations larger than the detection limits (Table 10) are As, B, Ba, Ca, Cu, Mg, Na, Si and Sr. These average concentrations have average deviations of ±5 units in the last digit of the listed numbers. The boron, barium and silicon concentrations in the samples stored in Pyrex containers were 2.25 ppm (B), 0.13 ppm (Ba) and 15.5 ppm (Si), significantly higher than in the samples stored in Cubitainers. According to information supplied by the Wheaton Glass Co., the manufacturer of the Pyrex containers, a soda ash-barium-borosilicate melt was used in the manufacture of the containers. The sodium concentration averaging 233±3 ppm was the same in all four samples.

No significant changes in arsenite, arsenate and total arsenic concentrations were observed during the one week period, during which the initial analyses were performed.

Delta Water Samples

The results for the Delta water samples are summarized in Table 8 (total arsenic concentrations), Table 9 (arsenite and arsenate concentrations) and Table 11 (trace elements by ICP). The total arsenic concentration is approximately 21 ppb. Arsenite and arsenate concentrations are comparable in magnitude and close to the detection limits of the methods employed. According to ICP results (Table 11) Ba, B, Ca, Cu, Fe, Mg, Na, Pb, Si and Sr are present in the water samples. The Ba and B concentrations in the samples stored in glass containers are again higher than those of samples kept in plastic containers. The Delta water samples are harder

TABLE 9. CONCENTRATIONS OF ARSENITE AND ARSENATE IN ppb IN THE HINCKLEY AND DELTA WATER SAMPLES

Location	Ascorbic Acid	Plastic Container	Pyrex Container	HG**		DPP**	
				Arsenite	Arsenate	Arsenite	Arsenate
Hinckley	-	-	+	4.7±0.2*	174±6*	--	189±10*
	+	-	+	23±7*	177±9*	--	200±22*
	-	+	-	4.7±0.1†	180±7†	12±1†	179±10†
	+	+	-	25±5†	158±6†	11±1†	179±20†
Delta	-	-	+	4±2*	14±2*	--	23±7*
	+	-	+	14±3*	5±1*	--	19±2*
	-	+	-	9±1†	9±1†	--	--
	+	+	-	11±1†	7±1†	--	--

* Average ± average deviation of three determinations each on four samples.

†Average ± average deviation of three determinations on one sample.

**
HG: Hydride generation.
DPP: Differential pulse polarography.

TABLE 10. TRACE ELEMENT CONCENTRATIONS IN THE HINCKLEY WATER SAMPLES DETERMINED BY ICP†

xxxx ······ Detection Limit in ppb

E

Concentration of the Element in ppm Found in the Sample* ······ yyyy

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li	0.46 Be											9 B 1.2	C	N	O	F	Ne
5.5 Na 233	34 Mg 1.83											50 Al	16 Si 13.4	150 P	S	Cl	Ar
42 K	45 Ca 3.24	8 Sc	16 Ti	8 V	8 Cr	1 Mn	8 Fe	6 Co	10 Ni	4 Cu 0.09	10 Zn	Ga	Ge	57 As 0.165	84 Se	Br	Kr
Rb	0.44 Sr 0.10	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba 0.026	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

---

†The average deviation of the listed concentrations is ±5 units of the last digit.

TABLE 11. TRACE ELEMENT CONCENTRATIONS IN THE DELTA WATER SAMPLES DETERMINED BY ICP[†]

xxxx
E
yyyy

xxxx ...... Detection Limit in ppb

yyyy ...... Concentration of the Element in ppm Found in the Sample*

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li	0.46 Be											9 B 0.01	C	N	O	F	Ne
5.5 Na 65	34 Mg 6.65											50 Al	16 Si 15.3	150 P	S	Cl	Ar
42 K	45 Ca 15.4	8 Sc	16 Ti	8 V	8 Cr	1 Mn	8 Fe 0.007	6 Co	10 Ni	4 Cu 0.26	10 Zn	Ga	Ge	57 As	84 Se	Br	Kr
Rb	0.44 Sr 0.52	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba 0.045	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb 1.17	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

[†]The average deviation of the listed concentrations is ±5 units of the last digit.

(15 ppm Ca, 6.6 ppm Mg) than the Hinckley samples. The sodium concentration is 65 ppm. The relatively high Pb (1.3 ppm) and Sr (0.5 ppm) concentrations are noteworthy.

Organic arsenic compounds were not detected in the Delta water samples.

WATER SAMPLES FROM ANTOFAGASTA, CHILE

Water samples from the drinking water supply for the city of Antofagasta, Chile were collected by Dr. K. J. Irgolic at the water treatment plant on October 21, 1980. Three samples were taken from the untreated water just before it entered the treatment plant. The faucet attached to the large delivery pipe was opened and water was allowed to run for five minutes. Then the one-quart plastic Cubitainers were rinsed with the water, filled to the brim and closed tightly. No air was left in the container. One of these samples was preserved with 0.1% by weight of ascorbic acid. Two samples were similarly taken from the pipe leaving the water treatment plant. All samples were collected in plastic Cubitainers. The analyses were performed during the period October 25 to November 10, 1980.

The following analytical methods were employed: graphite furnace atomic absorption spectrometry (GFAA), differential pulse polarography; hydride generation and inductively coupled argon plasma emission spectrometry.

The results of the total arsenic analyses on the five water samples from Antofagasta are summarized in Table 12. The average arsenic concentration (average deviation) obtained from all values for the untreated samples listed in Table 12 is 0.9±0.1 ppm. This value is close to the concentration of 0.8 ppm reported earlier for this water (69). The results for the untreated samples with and without ascorbic acid as preservative are in good agreement when the values obtained with a particular method or instrument are considered. The ICP analyses were carried out on three different instruments and with three different standards. The treated water samples gave arsenic concentrations in the range of 0.25 ppm to 0.51 ppm (Table 12). After passing through the alum treatment for arsenic removal the treated water has generally an arsenic concentration of approximately 80 ppb (70). The high arsenic values in the treated water samples collected at Antofagasta on October 21, 1980 could have been caused by a malfunction of the water treatment plant. The removal of arsenic by alum coagulation is highly pH dependent. The percent arsenic removed from arsenite-containing, chlorinated well water and from arsenate-containing tap water decreased from 90 percent at pH 7 to approximately 20 percent at pH 8.5 (70a). The treated water sample could have had a pH above 7. The pH values of the treated and untreated samples after nine months of storage in Cubitainers were 7.8 and 8.2, respectively. Another cause for the high arsenic values of the treated water samples could have been a local region of trapped arsenic-rich water or soluble deposit in the vicinity of the faucet from which the samples were taken.

TABLE 12. TOTAL ARSENIC CONCENTRATIONS IN ppm IN THE ANTOFAGASTA WATER SAMPLES

Sample	Ascorbic Acid	HG*†	GFAA*†	DPP*†	ICP*†
Untreated I	No	0.71±0.02	0.75±0.02	0.78±0.03	0.90, 0.78, 1.06
Untreated II	No	-	0.73±0.02	0.78±0.03	0.91, 0.81, 1.02
Untreated	Yes	0.72±0.06	0.70±0.02	-	0.91, 0.82, 1.05
Treated I	No	0.43±0.02	0.40±0.01	0.41±0.01	0.51, 0.42, 0.33
Treated II	No	-	0.36±0.01	-	0.32, 0.33, 0.25

† HG: Hydride generation.
DPP: Differential pulse polarography.
ICP: Inductively coupled argon plasma emission spectrometry.
GFAA: Graphite furnace atomic absorption spectrometry.

* Average ± average deviation of at least three determinations for each sample.

The arsenic compounds present in the Antofagasta water samples were determined by the hydride generation technique and by differential pulse polarography. Most of the arsenic is in the form of arsenate (Table 13). The arsenite concentrations in the preserved and unpreserved, untreated water samples are at most 16 ppb. The treated water sample contained 3 ppb arsenite. No methylated or other arsenic compounds were detected.

TABLE 13. CONCENTRATIONS OF ARSENITE AND ARSENATE IN ppm IN THE ANTOFAGASTA WATER SAMPLES

Sample	Ascorbic Acid	Arsenate**	Arsenite**
Untreated	No	0.70±0.02* 0.78±0.03†	0.016±0.001* -
Untreated	Yes	0.70±0.02* 0.78±0.03†	0.016±0.001* -
Treated	No	0.43±0.02* 0.41±0.01†	0.003±0.001* -

* Hydride generation method † Differential pulse polarography
**Average ± average deviation from at least three determinations given.

Trace elements in the Antofagasta water samples were determined by inductively coupled argon emission spectrometry employing three different instruments. Among the 41 elements on the analytical arrays of these instruments Li, Na, K, Mg, Ca, Sr, Ba, Fe, B, Al, Si, P, and As were present in the untreated (Table 14) as well as the treated (Table 15) water samples above the detection limits, which are also listed in these Tables. The values obtained for Mn and Cu are very close to the detection limits. Selenium was not found in the samples (detection limit 84 ppb). The beryllium content (7.4 ppm) of the untreated samples is noteworthy. Beryllium is a proven carcinogen (70b, c), and beryllium sulfate has been shown to cause enhancement of SA7 transformation of fetal cells of the Syrian hamster (70d). Additional analyses for beryllium in the untreated water samples should be carried out. The treated water samples did not contain beryllium.

The trace element concentrations obtained for the untreated, not preserved, samples did not differ from those found in the untreated sample preserved with 0.1% by weight of ascorbic acid with the exception of the values for Fe and Mn. Therefore the nine values available for each of the other elements were averaged. These average values with their average deviations are listed in Table 14. The Fe and Mn concentrations in the

TABLE 14. TRACE ELEMENT CONCENTRATIONS IN THE UNTREATED ANTOFAGASTA WATER SAMPLES DETERMINED BY ICP†

xxxx	……Detection Limit in ppb
E	
yyyy	Concentration of the Element in ppm Found in the Sample*

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li .62/2	0.46 Be 7.4/2											9 B 2.8/2	C	N	O	F	Ne
5.5 Na 102/2	34 Mg 7.4/2											50 Al .08/3	16 Si 38.7/6	150 P .34/3	S	Cl	Ar
42 K 13.3/7	45 Ca 20.0/2	8 Sc	16 Ti	8 V	8 Cr	1 Mn .002	8 Fe .11/2	6 Co	10 Ni	4 Cu .003	10 Zn	Ga	Ge	57 As .91/8	84 Se	Br	Kr
Rb	0.44 Sr .28/2	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba 008/1	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

†Average concentrations/average deviations are given in the form 20.0/2 to be read as 20.0±0.2.

TABLE 15. TRACE ELEMENT CONCENTRATIONS IN THE TREATED ANTOFAGASTA WATER SAMPLES DETERMINED BY ICP[†]

xxxx ...... Detection Limit in ppb
E
yyyy ...... Concentration of the Element in ppm Found in the Sample*

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li .64/1	0.46 Be											9 B 2.8/1	C	N	O	F	Ne
5.5 Na 103/1	34 Mg 7.5/2											50 Al 3.1/8	16 Si 363/9	150 P .26/5	S	Cl	Ar
42 K 132/8	45 Ca 203/4	8 Sc	16 Ti	8 V	8 Cr	1 Mn .006	8 Fe .3/1	6 Co	10 Ni	4 Cu .007	10 Zn	Ga	Ge	57 As .36/7	84 Se	Br	Kr
Rb	0.44 Sr .29/2	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba 008/1	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

[†] Average concentrations/average deviations are given in the form 20.0/2 to be read 20.0±0.2.

untreated samples with ascorbic acid were five- and twenty-times higher, respectively, than the values in the untreated samples without ascorbic acid. It could be that Fe and Mn were introduced by the ascorbic acid.

Another significant difference between the trace element concentrations in the untreated and treated water samples was noted for aluminum and arsenic. The untreated water had 0.08 ppm Al and 0.91 ppm As, whereas the treated water gave 3.1 ppm Al and 0.36 ppm As. The water treatment process uses potassium aluminum sulfate to produce aluminum hydroxide. The higher aluminum concentration in the treated water is, therefore, not unexpected.

Another set of samples from Antofagasta had been received by mail from the EPA Laboratories in Cincinnati during October 1977. The six samples were provided by Dr. Jane L. Valentine, Assistant Professor of Public Health, University of California, Los Angeles. The samples had been collected sometime before August 30, 1977. The exact collection date was not stated in the transmittal letter. The samples were received in thin-walled polyethylene containers (Cubitainers) which were closed by polyethylene caps with paper liners. All samples were colorless and contained little if any suspended matter. All samples were filtered through 0.45 μm Millipore filters. The filtrates were stored in 250 mL acid-cleaned polypropylene bottles in a refrigerator after acidification with the acids listed in Table 16. The analyses of the samples began on October 14, 1977.

The results of total element analysis for As, Na, Fe, Mn, Zn and Cu by atomic absorption spectrometry are summarized in Table 16.

TABLE 16. RESULTS OF TOTAL ELEMENT ANALYSIS FOR WATER SAMPLES FROM ANTOFAGASTA, CHILE COLLECTED AND ANALYZED IN 1977

Sample	2 mL Conc. Acid Added	As* ppm	Na** ppm	Fe** ppm	Mn** ppm	Zn** ppm	Cu** ppm
Untreated	H_2SO_4	0.52	54	0.18	†	0.042	†
	HNO_3	0.89	106	0.18	†	0.10	†
	none	0.91	106	0.15	†	0.047	†
	$HClO_4$	0.91	106	0.55	†	0.058	†
Treated	HNO_3	0.088	107	†	†	0.10	†
	H_2SO_4	0.081	107	†	†	0.10	†

* Graphite furnace atomic absorption spectrometry.
** Flame atomic absorption spectrometry.
† Less than the detection limit (Fe 0.075 ppm, Mn 0.075 ppm, Cu 0.10 ppm).

The total arsenic concentrations in the untreated Antofagasta water samples ranged from 0.5 to 0.9 ppm as determined by flameless atomic absorption spectrometry, in good agreement with the values found in the samples collected in 1980. The two samples which had passed through the water treatment plant had an arsenic concentration of 0.08 ppm in agreement with the values obtained in Antofagasta (70).

Arsenite and arsenate were determined only in the sample which had not been acidified. Only arsenite (0.12 ppm) and arsenate (0.80 ppm) were detected with no evidence for organic arsenic compounds. These arsenate and arsenite determinations were carried out by differential pulse polarography employing pyrogallol as the reagent to make arsenate electroactive. These values are considered less reliable than the ones obtained with the hydride generation technique (see Table 13).

WATER SAMPLES FROM ALASKA

Water samples from Alaska with arsenic concentrations in the ppm range were collected and shipped to College Station by air freight on October 17, 1979 and July 29, 1980 by the Alaska Department of Environmental Conservation in Fairbanks, Alaska. The samples came from the Barefoot and Mauer sites. The samples were collected in Pyrex containers and plastic Cubitainers. Ascorbic acid or nitric acid were added to some samples for preservation purposes. The preserved samples were clear without any sign of precipitates. The unpreserved samples contained orange-brown precipitates characteristic of iron(III) hydroxide. The analyses were performed during the two-week period following receipt of the samples.

The following analytical methods were employed: hydride generation, differential pulse polarography, inductively coupled argon plasma emission spectrometry and graphite furnace atomic absorption spectrometry.

The results of the total arsenic analyses and the determination of arsenite and arsenate are summarized in Table 17. The analyses of the Barefoot samples collected on 7/28/80 gave total arsenic concentrations of 3.3±0.1 ppm not including the DPP values. Although the DPP results are in agreement with the values obtained by ICP and HG, their large average deviations makes them rather unreliable. The analyses of the 1979 Barefoot samples produced average total arsenic concentrations of 2.9±0.2 ppm by ICP. The much higher (6.8 ppm) values obtained by DDP are suspect. Arsenic could not be determined by the hydride generation method because of instrumental problems.

The more reliable 1980 analyses for arsenite and arsenate show that approximately 70 percent of the total arsenic is present as arsenite. The sample preserved with ascorbic acid gave the same results as the sample treated with nitric acid (Table 17).

Tables 18 and 19 report the results of trace element analyses on the two sets of Barefoot samples by ICP. The elements present above the detection limits were: Li, Na, K, Mg, Ca, Sr, Ba, Ti, V, Cr, Mn, Fe, Cu,

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TABLE 17. RESULTS OF TOTAL ARSENIC ANALYSES AND ARSENITE AND ARSENATE DETERMINATIONS IN ALASKA WATER SAMPLES **

Sample	Glass	Plastic	Ascorbic Acid 0.1%	0.1 M HNO_3	Total Arsenic Concentration by				
					Hydride Generation	DPP	ICP	Arsenite	Arsenate
Barefoot 10/17/1979	+	-	-	-	-	6.6	2.7	2.3†	4.3†
	+	-	+	-	-	6.8	3.1	2.4†	4.4†
	-	+	-	-	-	6.7	2.6	2.3†	4.4†
	-	+	+	-	-	6.9	3.2	2.4†	4.5†
Barefoot 7/28/1980	-	+	-	+	3.05±0.06	3.2±0.7	3.4±0.1	2.4±0.1*	0.7±0.1*
								2.5±0.7	0.7±0.1
	-	+	+	-	3.26±0.09	3.0±0.9	3.4±0.1	2.4±0.1*	0.8±0.1*
Mauer 10/17/1979	+	-	-	-	-	4.6	4.4±0.1	4.5	0.1
	+	-	+	-	-	-	4.6±0.1	3.7	0.2
	-	+	-	-	-	4.7	-	3.8	0.2
	-	+	+		-	4.0	4.4±0.1	4.6	0.1
Mauer 7/28/1980	-	+	-	+	5.4±0.2	6.5±0.4	5.9±0.1	4.5±0.4*	1.0±0.4*
	-	+	+	-	4.6±0.2	5.5±0.2	6.1±0.1	0.35±0.02*	4.3±0.2*

* Hydride generation method. † Differential pulse polarography.

**Average ± average deviation from at least three determinations per sample.

TABLE 18. TRACE ELEMENT CONCENTRATIONS DETERMINED BY ICP IN THE BAREFOOT WATER SAMPLES FROM ALASKA† (COLLECTED IN 1979)

xxxx ......Detection Limit in ppb
E
Concentration of the ......yyyy
Element in ppm Found
in the Sample*

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li	0.46 Be											9 B	C	N	O	F	Ne
5.5 Na 47/2	34 Mg 113/1											50 Al <0.06	16 Si 11/1	150 P	S	Cl	Ar
42 K	45 Ca 291/8	8 Sc	16 Ti 008/2	8 V .15/1	8 Cr .033/7	1 Mn .44/4	8 Fe 53/5	6 Co	10 Ni	4 Cu .010/6	10 Zn .14/8	Ga	Ge	57 As 2.9/3	84 Se	Br	Kr
Rb	0.44 Sr .47/3	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd .010/5	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba .23/1	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

†Average concentrations/average deviations are given in the form 20.0/2 to be read as 20.0±0.2.

TABLE 19. TRACE ELEMENT CONCENTRATIONS DETERMINED BY ICP IN THE BAREFOOT WATER SAMPLES FROM ALASKA[†] (COLLECTED IN 1980)

xxxx ······ Detection Limit in ppb

E

Concentration of the Element in ppm Found in the Sample* ······ yyyy

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li	0.46 Be											9 B	C	N	O	F	Ne
5.5 Na 37/1	34 Mg 52/1											50 Al .50/3	16 Si ND	150 P .27/9	S 28/1	Cl	Ar
42 K 12/1	45 Ca 180/9	8 Sc	16 Ti	8 V	8 Cr	1 Mn .40/1	8 Fe 29/1	6 Co	10 Ni	4 Cu	10 Zn	Ga	Ge	57 As 3.4/1	84 Se	Br	Kr
Rb	0.44 Sr ND	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba .17/2	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu

Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

[†] Average concentrations/average deviations are given in the form 20.0/2 to be read 20.0±0.2.
ND = Not determined.

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Zn, Cd, Al, Si, P and As. Comparison of Table 18 (results for samples collected in 1979) with Table 19 (results for samples collected in 1980) shows considerable differences between the concentrations of Na, Mg, Ca, Fe and Al in the two samples. Since some concentrations in the 1979 samples are higher and others lower than in the 1980 samples a systematic instrument-related error is unlikely. Errors in preparing standards should not be so frequent. The possibility that the composition of the water supply changed must be considered and should be checked on additional samples.

Three of the Barefoot samples collected in 1980 (one preserved with ascorbic acid, two preserved by acidification to 0.1 $\underline{M}$ HNO_3) were analyzed again by ICP eight months after collection. The analyses of samples preserved with nitric acid agreed fairly well with the results obtained shortly after the samples had been collected. The sample preserved with ascorbic acid gave considerable lower values for As, Ca, P and Fe.

The Mauer samples contain 5.1±0.7 ppm of arsenic when all the values in Table 17 are averaged. Arsenite is the predominant form. The arsenite concentration of one of these samples collected in a Cubitainer and preserved by ascorbic acid (bottom line in Table 17) found by the hydride generation technique was much lower than for the other samples. Repetition of these analyses produced the same value.

Trace element analyses by ICP (Tables 20 and 21) showed that Na, K, Mg, Ca, Sr, Ba, Ti, V, Cr, Mn, Fe, Zn, Al, Si and As were present. Differences similar to the ones pointed out for the Barefoot samples were noted between the trace element concentrations of the Mauer samples collected and analyzed one year apart. Analyses of the eight months old nitric acid-preserved samples gave results in general agreement with those reported in Table 21. The arsenic concentration in the ascorbic acid-preserved samples was 0.7 ppm, one-fourth of the value found in the samples acidified with nitric acid.

Samples collected in 1979, which had been preserved with ascorbic acid and had not been opened since the collection, had formed a black precipitate after two to three weeks. Similar samples in containers, which had frequently been opened for removal of aliquots for analyses, did not form this black precipitate. More than 90 percent of the arsenic originally in solution was present in the black precipitate. It is possible that these samples contained microorganisms which grew in the ascorbic acid medium reducing sulfur compounds to sulfide. Sulfide then would react with iron to produce black iron sulfides. Arsenic sulfides would coprecipitate with the iron compound. Approximately 20 ppm sulfur were found in these water samples by an ICP analysis.

WATER SAMPLES FROM TAIWAN

Two sets of water samples from Taiwan were analyzed. One set collected by Dr. Tseng, Department of Medicine, National Taiwan University, Taipei, arrived in College Station toward the end of May 1977. These samples came from wells at Pu-Tai and Pei-Men. The second set was collected

TABLE 20. TRACE ELEMENT CONCENTRATIONS DETERMINED BY ICP IN THE MAUER WATER SAMPLES FROM ALASKA†
(COLLECTED IN 1979)

xxxx ……Detection Limit in ppb
E
Concentration of the Element in ppm Found in the Sample* ……yyyy

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li	0.46 Be											9 B	C	N	O	F	Ne
5.5 Na 50/2	34 Mg 139/9											50 Al .06	16 Si 10.8/6	150 P	S	Cl	Ar
42 K 118/15	45 Ca 309/5	8 Sc	16 Ti .009/2	8 V .17/2	8 Cr .03	1 Mn .76/10	8 Fe .63/3	6 Co	10 Ni	4 Cu	10 Zn .3/1	Ga	Ge	57 As 4.5/1	84 Se	Br	Kr
Rb	0.44 Sr .60/3	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba .35/2	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

† Average concentrations/average deviations are given in the form 20.0/2 to be read 20.0±0.2.

TABLE 21. TRACE ELEMENT CONCENTRATIONS DETERMINED BY ICP IN THE MAUER WATER SAMPLES FROM ALASKA†
(COLLECTED IN 1980)

xxxx ...... Detection Limit in ppb
E
Concentration of the Element in ppm Found in the Sample* ...... yyyy

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li .007/1	0.46 Be											9 B	C	N	O	F	Ne
5.5 Na 35/1	34 Mg 55/1											50 Al .54/1	16 Si	150 P	S 17.5/3	Cl	Ar
42 K	45 Ca 200/9	8 Sc	16 Ti	8 V	8 Cr	1 Mn .56	8 Fe 25/1	6 Co	10 Ni	4 Cu	10 Zn	Ga	Ge	57 As 6.0/1	84 Se	Br	Kr
Rb	0.44 Sr	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba .29/1	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

†Average concentrations/average deviations are given in the form 20.0/2 to be read 20.0±0.2.

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by Dr. Fung-Jou Lu, Associate Professor of Biochemistry, College of Medicine, Taipei in the Yenshei-Province from two wells and shipped by air mail to College Station on July 9, 1980.

Pei-Men and Pu-Tai Samples

The samples from Pei-Men and Pu-Tai were received toward the end of May 1977 in Chlorox containers. Later, a second set of Taiwan samples was received in one gallon Cubitainers with the date of collection unknown. The samples had a yellowish hue with some flocculated matter present. Analysis of the Taiwan waters was initiated on June 24, 1977. All analyses were performed on the original, unfiltered, unpreserved water as received except that in some cases dilution with water from a sub-boiling still or with 0.1 N Ultrex HNO_3 was necessary to bring the analyte concentration into the linear region of the analytical working curve. Data relating to the speciation of arsenic, total arsenic, sodium, copper, manganese, zinc, and iron concentrations are given in Table 22. Tseng *et al*. (71) reported that the total arsenic concentrations for drinking water taken from artesian wells in 37 villages located in the southwest of Taiwan fell within the range 0.017 to 1.097 ppm. The total arsenic concentrations, determined by flameless atomic absorption spectrometry and neutron activation analysis for the Pei-Men and Pu-Tai samples, fell within this range. Only arsenite (∿0.07 ppm) and arsenate (∿0.57 ppm) could be detected. Since some of the well waters are known to be anaerobic, one can expect substantial chemical changes to have occurred in the samples since the time of collection. The sum of the arsenite and arsenate concentrations is somewhat less than the total arsenic level found by flameless AAS. The sodium concentration is

TABLE 22. ANALYSIS RESULTS FOR THE PEI-MEN AND PU-TAI WATER SAMPLES

Element		Pei-Men	Pu-Tai
Arsenite, ppm As	(Hydride technique)	0.05	0.09
Arsenate, ppm As	(Hydride technique)	0.52	0.63
Arsenite and Arsenate		0.57	0.72
total As, ppm	(GFAA)	0.72	0.76
total As, ppm	(Neutron activation)	0.76	-
Sodium, ppm	(Flame AAS)	282	223
Copper, ppm	(Flame AAS)	<<0.1	<<0.1
Manganese, ppm	(Flame AAS)	<<0.1	<<0.1
Zinc, ppm	(Flame AAS)	<<0.1	<<0.1
Iron, ppm	(Flame AAS)	<<0.1	<<0.1

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rather high (200-300 ppm).

Trace Elements in the Yenshei-Province Samples

The water samples from two wells (Well I and II) in the Yenshei-Province of Taiwan were collected in plastic Cubitainers. Two samples each of unpreserved water, preserved by addition of 0.1 weight percent of ascorbic acid and preserved by acidification to 0.1 M HNO_3 arrived by air mail at College Station on July 13, 1980 and were analyzed during the two week period following arrival.

The total arsenic concentrations and the concentrations of arsenite and arsenate in these samples are given in Table 23. The total arsenic concentrations determined by the hydride generation technique are in the range of 0.84 ppm to 1.1 ppm. Arsenate is the predominant arsenic compound in these samples. Unpreserved samples and samples preserved by addition of ascorbic acid had the same arsenite concentrations indicating that the chemical forms of arsenic in the samples did not change during the time period between collection and analysis.

The trace element concentrations in these water samples were determined by ICP shortly after arrival. The results, however, were unreliable. The arsenic concentrations in most of the samples were found to be <60 ppb by ICP. When our own ICP became operational the water samples were analyzed again. The arsenic concentrations in the eight month old samples were in fair agreement with those found by hydride generation in the freshly collected samples. The Yenshei water samples contained Li, Na, K, Mg, Ca, Sr, Fe, B, Si, P, As and S. The results of these analyses are summarized in Tables 24 and 25 for Well I and Well II, respectively. The chemical form of the sulfur in these water samples (0.5 and 1.5 ppm) was not determined. Sulfate was found in several well water samples from the blackfoot disease area in Taiwan (71a). Additional experiments are needed to determine the chemical form of sulfur. A special search should be made for sulfite, which caused decreased DNA synthesis in human lymphocytes and is mutagenic in microbial test systems (71b).

Fluorescent Compounds in the Yenshei Water Samples

Blackfoot disease is an affliction peculiar to the south-west coast of Taiwan. The disease is caused by blockage of the peripheral vascular system and extreme cases are identified by ulceration and loss of the limbs (72, 73). Fluorescent pollutants in artesian well waters have been cited as possible causes of this disease. As a result, several studies have been undertaken to identify and characterize these fluorescent compounds. Lu *et al*. (73) have found that the water produces an intense green fluorescence under ultraviolet light. Chloroform extracts of the water were separated into nine components by thin-layer chromatography. The pH of these waters gradually increased to 9.0 upon standing in air at room temperature or upon boiling. Attempts were made to isolate and identify the fluorescent compounds in the well waters (74). The compounds were adsorbed on an anion exchange resin and eluted with a 5% ammonium chloride solution. The eluate

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TABLE 23. RESULTS OF TOTAL ARSENIC ANALYSES AND ARSENITE AND ARSENATE DETERMINATIONS IN THE YENSHEI WATER SAMPLES*

Sample	Ascorbic Acid 0.1%	0.1 M HNO$_3$	Unpreserved	TOTAL ARSENIC, ppm HG	TOTAL ARSENIC, ppm ICP	Arsenite, ppm by HG	Arsenate, ppm by HG
Well I	-	+	-	0.84±0.02	0.73±0.01	-	-
	+	-	-	0.88±0.01	0.72±0.01	0.024±0.001	0.85±0.01
	-	-	+	0.85±0.01	0.73±0.01	0.022±0.001	0.83±0.08
Well II	-	+	-	1.05±0.08	0.95±0.01	-	-
	+	-	-	1.16±0.03	0.96±0.01	-	-
	-	-	+	1.11±0.02	0.98±0.01	0.024±0.01	1.08±0.02

*Average ± average deviation of at least three determinations per sample

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TABLE 24. TRACE ELEMENT CONCENTRATIONS IN THE YENSHEI WATER SAMPLES, WELL I, DETERMINED BY ICP

xxxx ······Detection Limit in ppb

E

Concentration of the Element in ppm Found in the Sample* ······ yyyy

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li 0.007	0.46 Be											9 B	C	N	O	F	Ne
5.5 Na 280	34 Mg 8.6											50 Al	16 Si 3.4	150 P 4.9	S 0.5	Cl	Ar
42 K 1.0	45 Ca 4.3	8 Sc	16 Ti	8 V	8 Cr	1 Mn	8 Fe 0.46	6 Co	10 Ni	4 Cu	10 Zn	Ga	Ge	57 As 0.73	84 Se	Br	Kr
Rb	0.44 Sr 0.08	2 Y	4 Zr	Nb	3 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															
				Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
				Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

TABLE 25. TRACE ELEMENT CONCENTRATIONS IN THE YENSHEI WATER SAMPLES, WELL II, DETERMINED BY ICP

xxxx ……Detection Limit in ppb

E

Concentration of the Element in ppm Found in the Sample* ……yyyy

*The absence of a yyyy figure indicates that the concentration is at or below the detection limit.

H																H	He
0.43 Li 0.01	0.46 Be											9 B 0.87	C	N	O	F	Ne
5.5 Na 196	34 Mg 23											50 Al	16 Si 3.4	150 P 2.3	S 1.5	Cl	Ar
42 K 13.6	45 Ca 17.5	8 Sc	16 Ti	8 V	8 Cr	1 Mn	8 Fe 0.50	6 Co	10 Ni	4 Cu	10 Zn	Ga	Ge	57 As 0.97	84 Se	Br	Kr
Rb	0.44 Sr 0.20	2 Y	4 Zr	Nb	8 Mo	Tc	Ru	Rh	Pd	2 Ag	6 Cd	55 In	120 Sn	32 Sb	103 Te	I	Xe
Cs	0.58 Ba	La	Hf	Ta	18 W	Re	Os	Ir	25 Pt	48 Au	32 Hg	91 Tl	84 Pb	49 Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	64 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

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was evaporated to dryness on a rotary evaporator at 50°. The residue was extracted with methanol and acetone. The extract produced six blue-green fluorescent bands on a TLC plate. One of the compounds was tentatively identified as either lysergic acid, dihydrolysergic acid or a related compound. Another study (75) has identified ergocalciferol as a contaminant in the drinking water. In addition to the chemical studies, a number of publications discuss the toxic (76, 77) and cytotoxic (78, 79) effects of the fluorescent compounds in the drinking water.

Since ergot alkaloids were suggested to cause the fluorescence in the artesian drinking water, ion-pair, reversed phase, high performance liquid chromatography was used in an attempt to identify the compounds. This method has been recently suggested for ergot alkaloids.

Experimental

Liquid chromatography was performed on an Altex/Beckman Model 312 MP High Performance Liquid Chromatograph. A Farrand Model 801 Spectrofluorometer equipped with a 10 μL quartz flow cell was used for fluorescence detection. For preparative liquid chromatography a 15 x 250 mm glass column was used. A Fisher Model 80 circulating water bath was employed to provide elevated temperature chromatography. Samples were concentrated on a Brinkman Rotavapor rotary evaporator.

Amberlite IRA-400C strong anion exchange resins, Partisil 10-SAX (strong anion exchange column), a Partisil 10-SCX (cation exchange column), reversed-phase columns (Altex), a 25 cm Spherisorb-C18 and a 15 cm Ultrasphere-ODS column were used for chromatography. Ergometrine, ergotamine tartrate, ergocryptine, ergocalciferol and D-lysergic acid were obtained from Sigma Chemical Co.

Results

First, the sample preparation procedure of Lu (74) was evaluated. The procedure requires the adsorption of the fluorescent compounds on an Amberlite anion exchange resin followed by extraction with methanol and then with acetone. One liter of unpreserved Yenshei Well I water, collected in a Pyrex bottle, was pumped through a preparative LC column packed with Amberlite IRA-400C using a solvent metering HPLC pump with a 2 μ fritted inlet filter. The Farrand fluorometer was used to monitor the column effluent for the appearance of fluorescent components. After about eight hours of pumping attempts were made unsuccessfully to elute the fluorescent components with aqueous 5% NH_4Cl, aqueous 10% NH_4Cl and aqueous 10% NH_4Cl/NH_4OH at pH 9. The dark brown band that had formed at the top of the column did not move. Ethanol, 1 M with respect to HCl, eluted most of the adsorbed components in the brown band as well as highly fluorescent resin decomposition products. The eluate was evaporated to dryness. Dark brown crystals were noted in the residue. During the extraction of the residue with MeOH the crystals dispersed but did not dissolve. Attempts at dissolving the brown crystals in water, acetone and cyclohexane failed.

A much simpler preparation procedure similar to that of Lu *et al*. (75), in which the filtered water is concentrated under vacuum at 50°, was adopted. The water was filtered through a cellulose filter disk with an average pore size of 0.5 μ. The filtrate was concentrated under vacuum at approximately 50° in a rotary evaporator. Typically 200 mL of well water were concentrated to 10.0 mL.

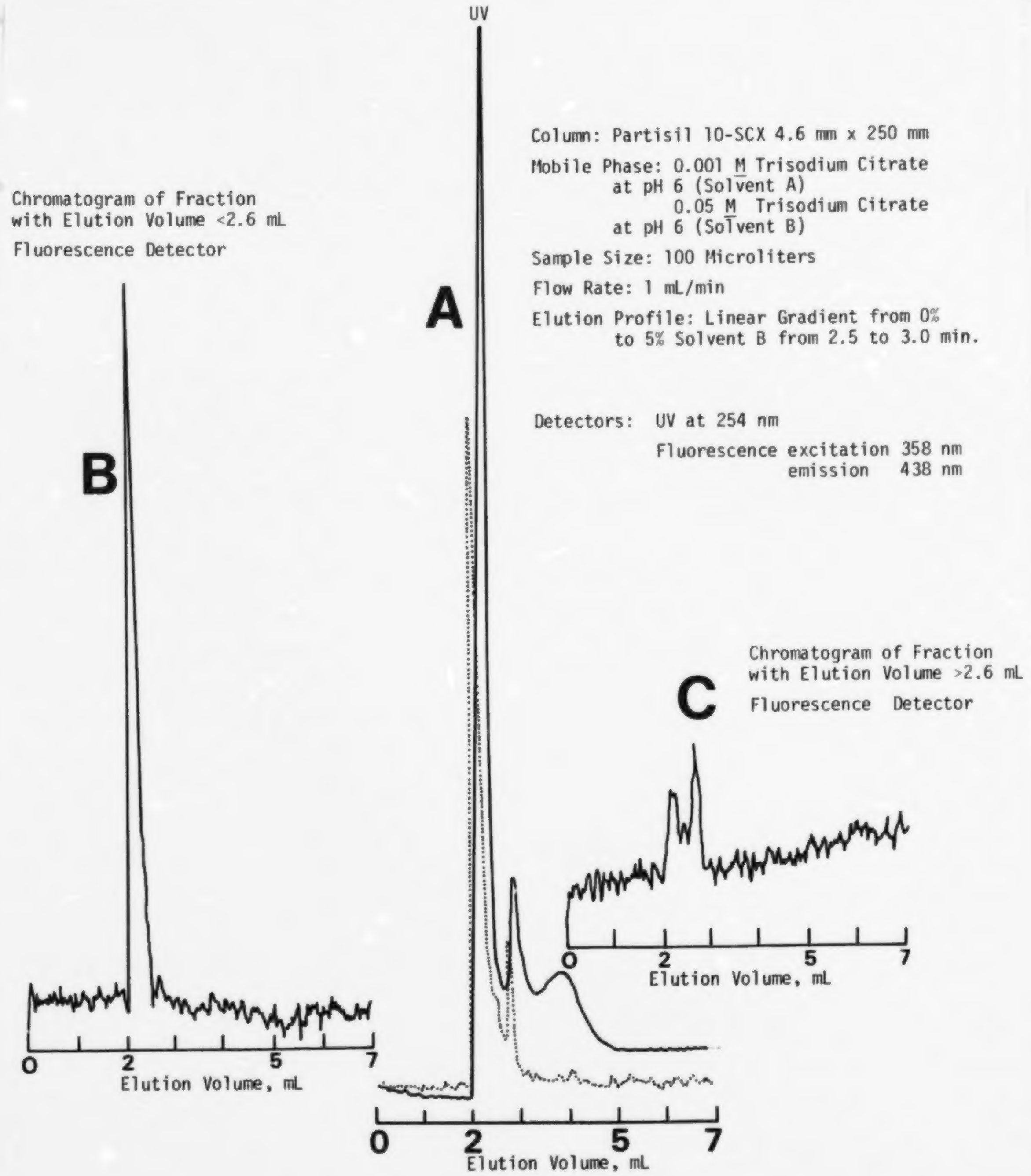
Chromatographic Analyses of Well Water Concentrates

High performance anion exchange chromatography employing a Partisil 10-SAX column and aqueous 0.15 *M* NH_4Cl at pH 3 or 0.1 M aqueous trisodium citrate at pH 6 as the mobile phases did not separate the fluorescent components. Washing the column with water and then methanol revealed that most of the injected sample had been tightly adsorbed to the column packing.

High performance cation exchange liquid chromatography using a Whatman Partisil 10-SCX column gave better results. Using gradient elution (0.001 *M* → .05 *M* sodium citrate at pH 6) two highly absorbing and fluorescent components and an additional nonfluorescent UV absorbing component were separated (Fig. 31A). Changing the ionic strength and/or the pH of the mobile phases did not improve the separation. The frequent appearance of a shoulder on the strongest fluorescence peak suggested the presence of an additional compound. Fractions up to 2.6 mL elution volume (Fig. 31B) and fractions with an elution volume >2.6 mL (Fig. 31C) were collected from four injections, concentrated and rechromatographed. A third fluorescent component was observed at an elution volume of 2.4 mL (Fig. 31C).

Next the feasibility of a reversed phase separation of the fluorescent compounds using ion-pair formation was investigated. Several experiments with hexanesulfonic acid and heptanesulfonic acid at pH 3 were conducted. Heptanesulfonic acid gave the best results. Figures 32 and 33 show chromatograms of the concentrated water samples and five alkaloid standards employing a UV-detector at 254 nm and a fluorescence detector, respectively. *D*-Lysergic Acid and ergometrine were not resolved under these conditions. Calciferol, a rather insoluble compound, does not fluoresce and, therefore, does not produce a signal when a fluorescence detector is employed (Fig. 33). The chromatograms contain also peaks arising from impurities in the aqueous solvent which fluoresce and are UV-detectable. Comparing the chromatograms of the alkaloid standards with the chromatograms of the water concentrates *D*-lysergic acid and/or ergometrine and ergotamine could be present in the water samples. The presence of calciferol cannot be ruled out. Ergotamine and a solvent impurity have the same elution volume with the impurity contributing approximately one third to the total area of the peak. The chromatograms of the water samples contain several peaks, which do not correspond to any of the five alkaloid standards.

The water samples, which had been concentrated to one twentieth of their original volume, deposited a white precipitate after approximately 36 hours. Such a sample was stored and analyzed after eight weeks to see whether any changes had taken place. The absorption and fluorescence chromatograms of the aged water sample are shown in Fig. 34. The



Column: Partisil 10-SCX 4.6 mm x 250 mm

Mobile Phase: 0.001 M Trisodium Citrate at pH 6 (Solvent A)
0.05 M Trisodium Citrate at pH 6 (Solvent B)

Sample Size: 100 Microliters

Flow Rate: 1 mL/min

Elution Profile: Linear Gradient from 0% to 5% Solvent B from 2.5 to 3.0 min.

Detectors: UV at 254 nm
Fluorescence excitation 358 nm
emission 438 nm

Chromatogram of Fraction with Elution Volume <2.6 mL
Fluorescence Detector

Chromatogram of Fraction with Elution Volume >2.6 mL
Fluorescence Detector

Figure 31. Cation exchange liquid chromatogram of the concentrated Yenshei water sample.

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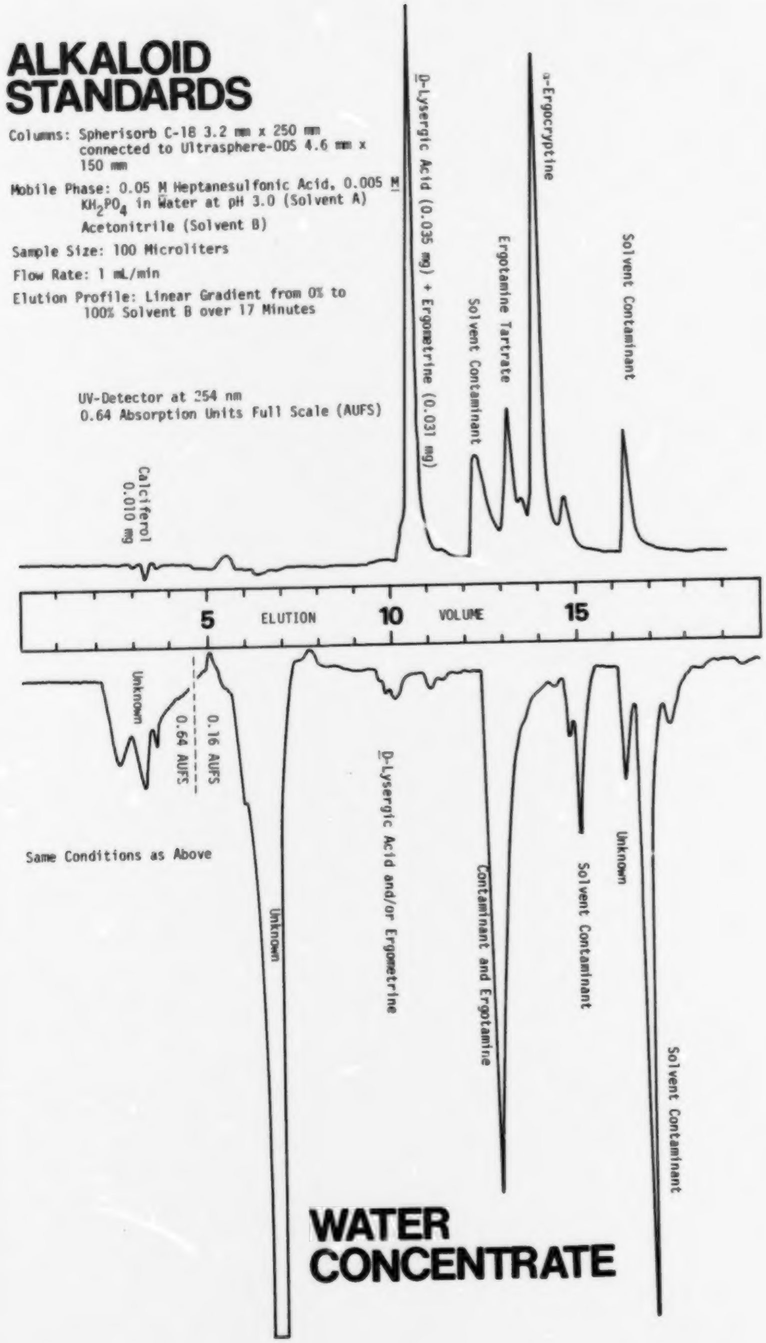

Figure 32. Ion-pair reversed phase chromatogram of the concentrated Yenshei water sample and alkaloid standards employing an UV-detector.

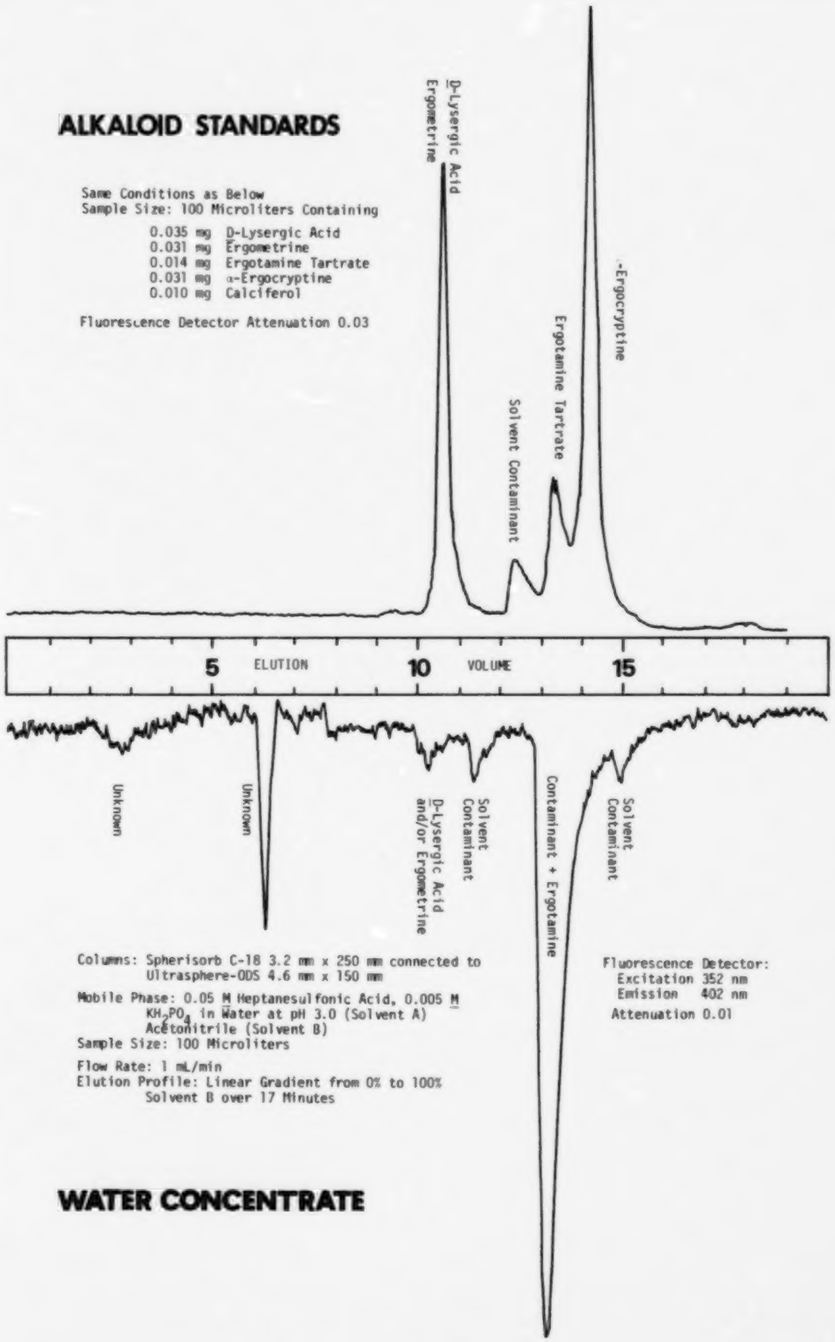

Figure 33. Ion-pair reversed phase chromatogram of the concentrated Yenshei water sample and alkaloid standards employing a fluorescence detector.

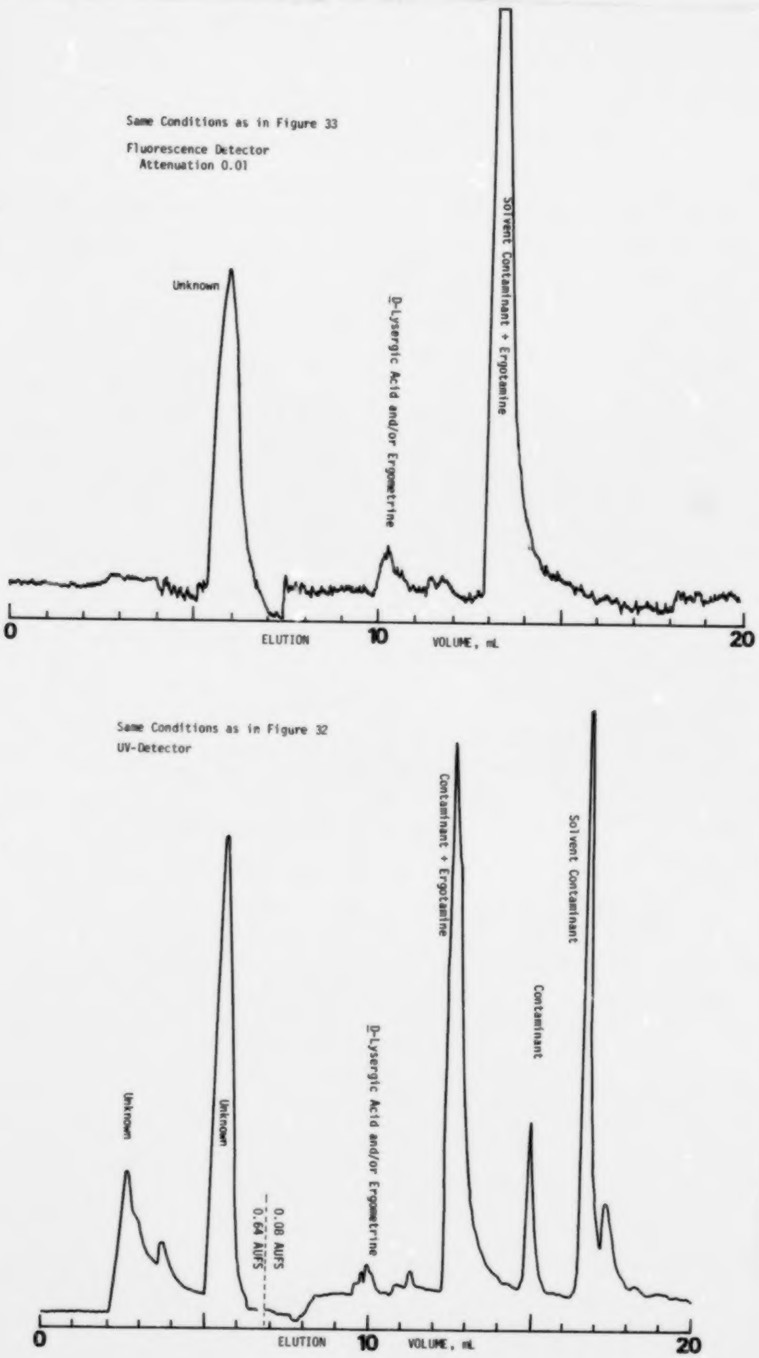

Figure 34. Ion-pair reversed phase chromatograms of the aged, concentrated Yenshei water sample employing a UV-detector at 254 nm and a fluorescence detector.

chromatograms of the aged sample are markedly different in the elution volume region from 5 to 7 mL and from 15 to 17 mL when compared to the chromatograms of the fresh samples (Fig. 32, 33).

Unconcentrated artesian well water was also analyzed by rapid scanning fluorometry as described previously (81). The emission-excitation matrix for this samples is shown in Figure 35. The intensity values represent the sum of four single frame scans electronically added to comprise a single integrated spectrum. The data indicate a blue-green fluorescence with the emission spread over more than 100 mn from ~350 nm to 500 nm. Rapid scanning fluorometry could be used for routine and quick characterization of well waters containing fluorescent compounds.

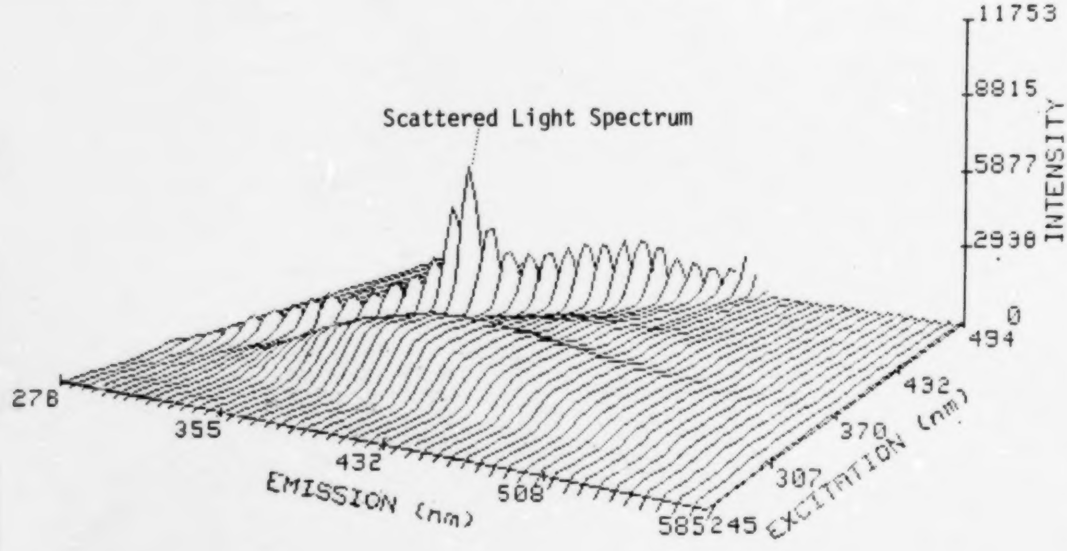

Figure 35. Rapid scanning fluorescence analysis of unconcentrated Yenshei well water.

Chromatography and analysis of the fluorescence of the Yenshei water sample showed that several alkaloids and other unknown fluorescent compounds were present. A sufficient amount of sample was not available to separate these compounds chromatographically and analyze them by mass spectrometry. Additional work in this area is urgently needed.

WATER SAMPLES FROM NOVA SCOTIA

The initial set of Nova Scotia samples was received in linear-polyethylene bottles. The second set was collected in one gallon Cubitainers at the same time (April 21, 1977). Sample 1 was taken from a dug well suspected of causing chronic arsenic poisoning and anemia. It contained a

TABLE 26. ANALYSIS RESULTS FOR THE NOVA SCOTIA WATER SAMPLES

Element	Sample 1 John Hartlin	Sample 2 H. Sullivan
Arsenite, ppm As		
6 weeks after collection*	0.68	0.05
at time of sample collection††	4.5	0.31
24 hrs. after collection††	-	0.21
Arsenate, ppm As		
6 weeks after collection*	4.48	0.23
at time of sample collection††	3.5	0.32
24 hrs. after collection††	-	0.18
Total Inorganic Arsenic (Arsenite and Arsenate)		
6 weeks after collection*	5.16	0.28
at time of sample collection††	8.0	0.63
24 hrs. after collection††	-	0.39
Total Arsenic (6 weeks after collection)		
AAS**	6.8	0.52
NAA††	8.2	1.1
Sodium, ppm	60	50
Copper, ppm	0.08	0.21
Manganese, ppm	2.4	<0.1
Zinc, ppm	<<0.1	0.9
Iron, ppm	<<0.1	<<0.1
Ammonia, ppm††	15	-
Oxygen, ppm††	<1.0	3.2
pH††	6.6	8.0

* Determined by Hydride Generation.

† Neutron Activation Analysis.

** Graphite Furnace Atomic Absorption Spectrometry.

†† Data provided by C. E. Tupper, Nova Scotia Department of Public Health.

substantial quantity of large particulate matter. Sample 2 was collected from a drilled well with no known harmful physiological effects.

The results of the arsenic speciation studies performed during June 1977 and total arsenic, sodium, copper, manganese, zinc, iron, ammonia, dissolved oxygen, and pH determinations are shown in Table 26. The total arsenic concentration in sample 1 is approximately 6 ppm. The arsenite and arsenate analyses carried out in the field by the Nova Scotia Department of Public Health indicated comparable concentrations for arsenite (4.5 ppm) and arsenate (3.5 ppm) in sample 1. The dissolved oxygen content for sample 1 was very low at less than 1 ppm. Six weeks after the collection the sample contained 0.68 ppm arsenite and 4.48 ppm arsenate. Since a considerable period of time had elapsed between sampling and analysis, aerial oxidation of the arsenite to arsenate could have occurred. The sum of the arsenite and arsenate levels is less than the total arsenic concentration determined by GFAA. The differences for samples 1 and 2 are 1.6 and 0.24 ppm, respectively. It is not certain whether these differences reflect changes in the total arsenic content as a function of time (decreasing with increasing time), are indicative of undetermined arsenic species or were caused by interferences specific for GFAA. The dissolved arsenic concentration in sample 2 dropped sharply after 24 hours from 0.63 ppm to 0.39 ppm with the remainder present as particulate arsenic (0.17 ppm).

An unusually high manganese content (2.4 ppm) was found for sample 1. Copper in the range 0.1 to 0.2 was also detected in both Nova Scotia samples. Zinc was found in sample 2 at the 0.1 ppm level. The sodium levels were quite moderate at ∿50 ppm.

DISCUSSION

The water samples analyzed as part of this project had total arsenic concentrations in the range 18 ppb to 8 ppm. The arsenite/arsenate ratios are in the range 0.007 to 3.4 (Table 27). No indications of the presence of methylated arsenic compounds, which are reducible to methylarsine or dimethylarsine, have been found. Experiments with the high pressure liquid chromatograph/graphite furnace atomic absorption spectrometer system, which would provide information about the presence of organic arsenic compounds not reducible to methylarsines, detected only arsenite and arsenate. Comparison of total arsenic concentrations with the sum of the arsenite and arsenate concentrations place an upper limit on the concentrations of any other arsenic compounds which might be present. These upper limits are in most cases in the low ppb range.

The other trace elements found in these water samples by ICP are listed in Table 27. There are no particularly offensive elements in these water supplies with exception of beryllium (7.4 ppm) in the untreated Antofagasta samples.

The various physiological effects observed in populations exposed to these arsenic-containing drinking water supplies (Table 28) could have been caused by the presence of varying amounts of arsenite and arsenate

TABLE 27. SUMMARY OF TOTAL ARSENIC, ARSENITE, ARSENATE AND TRACE ELEMENT CONCENTRATIONS IN DRINKING WATER SAMPLES†

	Hinckley	Delta	Barefoot ††	Mauer ††	Antofagasta Untreated	Antofagasta Treated	Yenshei I	Yenshei II	Nova Scotia 1	Nova Scotia 2
Total As	0.18	0.02	3.1	4.5-6.0	0.75	0.41**	0.85	1.1	8.0	0.63
Arsenite	0.010	0.010	2.4	0.35-4.6	0.016	0.003	0.023	0.024	4.5	0.31
Arsenate	0.18	0.010	0.7	0.1-4.3	0.74	0.41	0.84	1.08	3.5	0.32
Arsenite/ Arsenate Ratio	0.06	~1.0	3.4		0.02	0.007	0.03	0.02	1.3	1.0
Al	-	-	0.06-0.50	0.06-0.54	0.08	3.1	-	-	*	*
B	1.2	0.01	-	-	2.8	2.8	-	0.87	*	*
Ba	0.026	0.045	0.17	0.29	0.008	0.008	-	-	*	*
Be	-	-	-	-	7.4	-	-	-	*	*
Ca	3.24	15.4	180-291	200-309	20.0	20.3	4.3	17.5	*	*
Cu	0.08	0.26	-	-	0.003	0.007	-	-	0.008	0.21
Fe	-	0.007	29-53	25	0.11	0.30	0.46	0.50	<0.1	<0.1
K	-	-	12	13-118	13.3	13.2	1.0	13.6	*	*
Li	-	-	-	0.007	0.62	0.64	0.007	0.01	*	*
Mg	1.83	6.65	52-113	55-139	7.4	7.5	8.6	23	*	*
Mn	-	-	0.42	0.56-0.76	0.002	0.006	-	-	2.4	<0.1
Na	233	65	37-47	35-50	102	103	280	196	60	50
P	-	-	0.27	-	0.34	0.26	4.9	2.3	*	*
Pb	-	1.17	-	-	-	-	-		*	*
S	*	*	28	17.5	*	*	0.5	1.5	*	*
Si	13.4	15.3	11.0	11.0	38.7	36.3	3.4	3.4	*	*
Sr	0.10	0.52	0.47	0.60	0.28	0.29	0.08	0.20	*	*
Ti	-	-	0.008	0.009	-	-	-	-	<0.1	<0.9
V	-	-	0.15	0.17	-	-	-	-	*	*
Zn	-	-	0.14	0.30	0.10	0.10	-	-	*	*

* Not determined. ** As in treated water is normally less than 100 ppb. See text for discussion.

† The concentrations are given in ppm. †† Results from two different samples collected one year apart.

TABLE 28. ARSENIC-CONTAINING WATER SUPPLIES AND THEIR PHYSIOLOGICAL MANIFESTATIONS IN MAN

Sampling Location	Type of Water and Range of the Total Arsenic Concentration	Symptoms Observed in the Population
Taiwan (37 villages)	artesian well waters used for 45 years; arsenic leached from geologic deposits; 0.017-1.097 ppm; median 0.5 ppm.	melanois, keratosis, skin cancer; 15% prevalence among males over age 60, normal incidence 2-3%.
Chile (Antofagasta)	drinking water supply since 1958; 0.8 ppm before water treatment, 0.1 after water treatment.	melanosis, hyperkeratosis; vascular manifestations: myocardialischemia, hemipleglia with occlusion of the carotid artery, mesenteric arterial thrombosis, pneumonia.
Bakersfield, California	community drinking water supply; 0.3-0.7 ppm.	no adverse effects reported.*
Fallon, Nevada	drinking water, 0.1 ppm.	no known adverse physiological effects.*

*In a study (82) of the arsenic exposure of populations in Bakersfield, California, and Fallon, Nevada, by a questionnaire designed to elicit information about arsenic related symptoms and diseases very few symptoms were reported. The incidence of these symptoms was not significantly different from control populations not exposed to arsenic.

(Table 27). It is also conceivable that one or more of the trace elements present in the water supplies act in concert with arsenic to cause the observed effects. Many more samples need to be analyzed and the results of these analyses correlated with epidemiological studies before a definite statement can be made about the interactions of trace elements with arsenite or arsenate.

The chromatographic work on the fluorescent compounds in the Taiwan well waters strongly suggests the presence of alkaloids such as D-lysergic acid, ergometrine and calciferol. Additional experiments (preparative chromatography, mass spectrometry) could not be carried out because of insufficient sample.

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